A. M. A.
ARCHIVES OF
PATHOLOGY

EDITORIAL BOARD

GRANVILLE A. BENNETT, Chicago, Chief Editor

CHARLES E. DUNLAP, New Orleans
WILEY DAVIS FORBUS, Durham, N. C.
STUART LIPPINCOTT, Seattle
WILLIAM B. WARTMAN, Chicago
GEORGE H. WHIPPLE, Rochester, N. Y.
S. B. WOLBACH, Boston

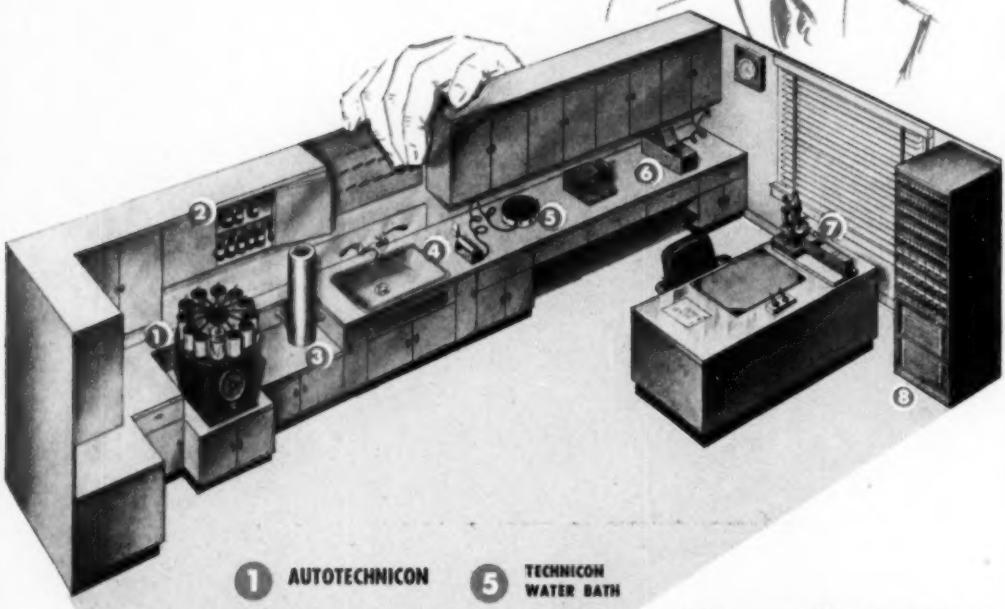
APRIL 1953
VOLUME 55 NUMBER 4

Published Monthly by
AMERICAN MEDICAL ASSOCIATION
535 NORTH DEARBORN STREET • CHICAGO 10, ILLINOIS

Entered as Second Class Matter Jan. 20, 1926, at the Postoffice at Chicago, Under the Act of March 3, 1879. Annual Subscription, $8.00

TABLE OF CONTENTS FIRST PAGE

more than a mere instrument— a completely integrated **system** for histologic preparation



1. AUTOTECHNICON
2. TECHNICON HISTOLOGIC REAGENTS
3. TECHNICON PIPETTE WASHER
4. TECHNICON PARAFFIN KNIFE
5. TECHNICON WATER BATH
6. TECHNICON MICROSLIDE DRYER
7. TECHNICON MICROSCOPE LAMP
8. LAB-AID SLIDE FILES

These are the elements of the Autotechnicon histologic processing system . . . each a notable technical contribution toward making the work of the pathological laboratory *easier*, with better, *surer* results.

Autotechnicon

THE TECHNICON COMPANY
215 East 149th Street, New York 51, N. Y.

CONTENTS

Original Articles

Acid Mucopolysaccharides of the Aorta PAGE

C. H. Bunting, M.D., and Henry Bunting, M.D., New Haven, Conn. 257

Effects of High Intensity Radiant Energy on Skin

G. E. Sheline, Ph.D., M.D.; E. L. Alpen, Ph.D.; P. R. Kuhl, A.B., and A. J. Ahokas, A.B., San Francisco 265

E. L. Alpen, Ph.D.; G. E. Sheline, Ph.D., M.D.; P. R. Kuhl, A.B., and A. J. Ahokas, A.B., San Francisco 280

Milk-Aspiration Pneumonia in Human and Animal Subjects

Thomas J. Moran, M.D., Pittsburgh 286

Developmental Brain Metabolism

Samuel P. Hicks, M.D., with the Technical Assistance of Alice A. Williams, Boston .. 302

Classification of Glandular Tumors of Salivary Glands

William H. Bauer, M.D., and John D. Bauer, M.D., St. Louis 328

Localizing Properties of Anti-Rat-Kidney Serum Prepared in Ducks

David Pressman, Ph.D.; Leonhard Korngold, Ph.D., New York, and Walter Heymann, M.D., Cleveland 347

Regular Departments

News and Comment 349

Books 352

THE A. M. A. ARCHIVES OF PATHOLOGY is published by the American Medical Association as a medium to advance pathology in the United States and to promote research and observation in this field.

Communications regarding subscriptions, reprints, etc., should be addressed, A. M. A. ARCHIVES OF PATHOLOGY, American Medical Association, 535 North Dearborn Street, Chicago 10.

Manuscripts for publication, books for review, and correspondence relating to contributions should be sent to Dr. Granville A. Bennett, Chief Editor, 1853 West Polk Street, Chicago 12, or to any other member of the Editorial Board.

Articles are accepted for publication on condition that they are contributed solely to the A. M. A. ARCHIVES OF PATHOLOGY. Manuscripts must be typewritten, preferably double spaced, and the original copy should be submitted. Zinc etchings and halftones will be supplied by the Association when the original illustrations warrant reproduction and when their number is not considered excessive.

Footnotes and bibliographies (the latter are used only in exhaustive reviews of the literature) should conform to the style of the *Quarterly Cumulative Index Medicus* and include, in the order given: name of author, title of article and name of periodical, with volume, page and year.

Matter appearing in the A. M. A. ARCHIVES OF PATHOLOGY is covered by copyright, but as a rule no objection will be made to its being reproduced in a reputable medical journal if proper credit is given. However, the reproduction for commercial purposes of articles appearing in the A. M. A. ARCHIVES OF PATHOLOGY or in any of the other publications issued by the Association will not be permitted.

The A. M. A. ARCHIVES OF PATHOLOGY is published monthly. The annual subscription price (for two volumes) is as follows: domestic, $8.00; Canadian, $8.40; foreign, $9.00, including postage. Current single copies, $1.00, postpaid, except special issues.

Checks, money orders and drafts should be made payable to the American Medical Association.

OTHER PERIODICAL PUBLICATIONS

of the American Medical Association

THE JOURNAL OF THE AMERICAN MEDICAL ASSOCIATION—Weekly. Covers all the medical sciences and matters of general medical interest. Illustrated. Annual subscription price (three volumes): domestic, $15.00; Canadian, $16.50; foreign, $19.00. Single copies, 45 cents.

A. M. A. ARCHIVES OF INTERNAL MEDICINE—Monthly. Devoted to the publication of advanced original clinical and laboratory investigations in internal medicine. Illustrated. Annual subscription price (two volumes): domestic, $10.00; Canadian, $10.40; foreign, $11.00. Single copies, $1.00.

A. M. A. ARCHIVES OF NEUROLOGY AND PSYCHIATRY—Monthly. A medium for the presentation of original articles on nervous and mental diseases, with abstracts from foreign and domestic literature, book reviews, transactions of special societies, etc. Illustrated. Annual subscription price (two volumes): domestic, $12.00; Canadian, $12.40; foreign, $13.50. Single copies, $1.25.

A. M. A. ARCHIVES OF DERMATOLOGY AND SYPHILOLOGY—Monthly. Devoted to advancing the knowledge of and progress in cutaneous diseases and syphilis. Publishes original contributions on these two subjects, transactions of the important dermatological societies, book reviews, etc. Illustrated. Annual subscription price (two volumes): domestic, $12.00; Canadian, $12.40; foreign, $13.50. Single copies, $1.25.

A. M. A. AMERICAN JOURNAL OF DISEASES OF CHILDREN—Monthly. Presents pediatrics as a medical science and as a social problem. Includes carefully prepared reviews, based on recent pediatric literature, abstracts from foreign and domestic literature, book reviews, transactions of special societes, etc. Illustrated. Annual subscription price (two volumes): domestic, $12.00; Canadian, $12.40; foreign, $13.50. Single copies, $1.25.

A. M. A. ARCHIVES OF SURGERY—Monthly. Devoted largely to the investigative and clinical phases of surgery, with monthly reviews on orthopedic and urologic surgery. Well illustrated. Annual subscription price (two volumes): domestic, $14.00; Canadian, $14.40; foreign, $15.50. Single copies, $1.25, except special numbers.

A. M. A. ARCHIVES OF OPHTHALMOLOGY—Monthly. Includes original articles on diseases of the eye, annual reviews of special subjects, book reviews, transactions of special societies, etc. Illustrated. Annual subscription price (two volumes): domestic, $12.00; Canadian, $12.40; foreign, $13.00. Single copies, $1.25.

A. M. A. ARCHIVES OF OTOLARYNGOLOGY—Monthly. A medium for the presentation of original articles on diseases of the ear, nose and throat, with abstracts from foreign and domestic literature, book reviews, transactions of special societies, etc. Illustrated. Annual subscription price (two volumes): domestic, $12.00; Canadian, $12.40; foreign, $13.00. Single copies, $1.25.

A. M. A. ARCHIVES OF INDUSTRIAL HYGIENE AND OCCUPATIONAL MEDICINE—Monthly. Devoted to the advancement of knowledge of the diseases of industry and to the publication of scientific investigation in this field. Illustrated. Annual subscription price (two volumes): domestic, $8.00; Canadian, $8.40; foreign, $9.00, including postage. Single copies, $1.00.

QUARTERLY CUMULATIVE INDEX MEDICUS—A complete subject and author index to the worth while current medical literature of the world. Issued twice a year. Volumes bound for permanent reference. Subscription price, calendar year: domestic, $20.00; Canadian, $22.00; foreign, $22.00.

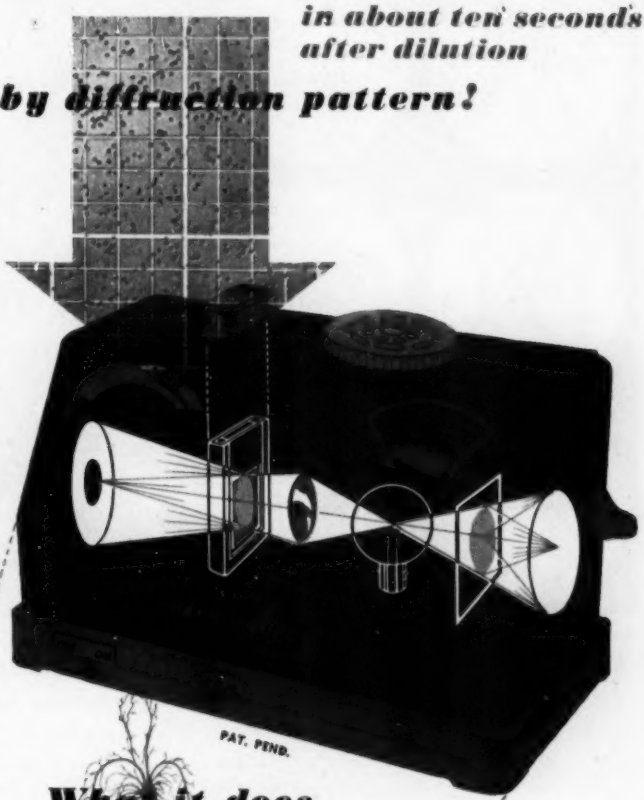
AMERICAN MEDICAL ASSOCIATION

535 North Dearborn Street CHICAGO 10

COUNT RED BLOOD CELLS

in about ten seconds after dilution

by diffraction pattern!



This is the story of the HAEMOSCOPE ...how it works, how it compensates, what it does. The Emil Greiner Company is proud to introduce you to this entirely new diagnostic instrument for the achievement of blood cell counts with remarkable precision, automatic compensation for variations in cell size and easy adaptation to existing laboratory procedure.

How it works A beam of light passes through a lens and cuvette containing a highly dilute suspension of blood. The directly transmitted light is focused upon the obscured portion of the annular photocell and therefore produces no current. The portion of the light which strikes the suspended blood cells is diffracted upon the active surface of the same photocell. The current so produced is measured against a standardized current produced by a secondary beam of light from the same light source which passes through a screen and diaphragm and strikes a secondary photocell.

Since the intensity of the diffracted light is a well known function of the number of blood cells, a calibrated scale has been devised to read directly.

How it compensates But the intensity of diffracted light is also a function of the cell size and shape as well as the number. Although larger cells produce a more intense light, less of it is made to strike the annular photocell, by carefully selecting its dimensions.

In this manner, the intensity of diffracted light is made practically independent of particle size and shape over a range of approximately 6 to 9 microns average cell diameter.

What it does

The HAEMOSCOPE, in its present form, provides:

1. An erythrocyte count with direct-readings in millions of red blood cells per cubic millimeter.
2. A haemoglobin determination in grams per hundred cubic centimeters.

On the erythrocyte count the HAEMOSCOPE will normally give readings to an accuracy of better than plus or minus ten per cent. Can also be adapted for research work on animals as well as human beings.

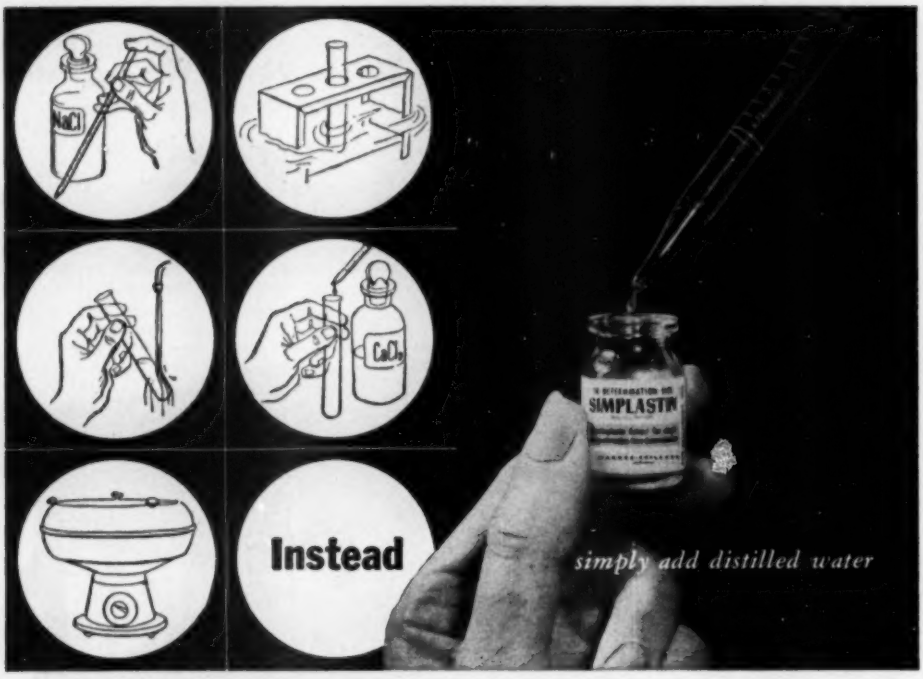
PRICE LIST

G5360 HAEMOSCOPE—complete with accessories, for immediate use, each.....$585

Send for Bulletin 3001, includes complete description, diagrams and directions for operation.

The EMIL GREINER Co.

20-26 N. MOORE STREET E&G Co. NEW YORK 13, N.Y.



simpler prothrombin time determinations

Accurate prothrombin time determinations are required in effective anticoagulant therapy. Simplastin insures greater accuracy, reduces the time factor from 45 to 5 minutes and requires only the addition of distilled water—in contrast to older, complicated methods. Sources of variability and dependence on technical dexterity are eliminated.

With Simplastin "the operator need only add a stated amount of distilled water and the material is ready for use."[1] Thus, "many costly and cumbersome steps are eliminated in preparation."[2] Results are readily reproducible.

Simplastin gives "considerable assurance and safety" to the clinician "who has hesitated to employ Dicumarol therapy because of the difficulty encountered in laboratory control."[1]

Simplastin makes available prothrombin time tests in which errors due to the human element in "extracting, centrifugation or filtering are entirely eliminated."[1]

For the laboratory, **Simplastin "simplifies the procedure . . ."[3]**

For the clinician, **Simplastin "eliminates sources of serious error"[3] in prothrombin time determinations.**

Available: 6-Determination Size and 20-Determination Size, both in boxes of 10 vials.

A trial supply and literature on request.

Bibliography: 1. Shapiro, S., et al.: Am. Heart J. *40*:766 (Nov.) 1950. 2. Schilling, F. J.; De Natale, A., and Mottram, F. C.: Am. J. M. Sc. 222:207 (Aug.) 1951. 3. Shapiro, S., and Weiner, M.: J. M. Soc. New Jersey *48*:52 (Feb.) 1951.

Simplastin®

WARNER-CHILCOTT *Laboratories,* NEW YORK

Paragon Tray Drawer Cabinet

Compact

U. S. Pat. No. 2,202,047
C101—Tray Drawer Cabinet for 3 x 1 Micro Slides
Capacity 4500—18¾ x 15¾ x 4¾

Low Cost

FOR FILING
MICROSCOPIC SLIDES 3 x 1"
KODACHROME TRANSPARENCIES
2 x 2" SLIDES
LANTERN SLIDES
(up to 3¼ x 4¼)
PETROGRAPHIC SLIDES

When you purchase a
PARAGON TRAY DRAWER CABINET YOU PURCHASE FILING SPACE ONLY NO WASTE SPACE-EVERY INCH USED

All Paragon Tray Drawer Cabinets are manufactured in standard sizes so that any number of sections may be interlocked to form one cabinet to accommodate any number of varied slides. The dimnsions of the different cabinets are the same as to length and width, varying only in height. The cabinet formed by interlocking may be 18¾ x 15¾; 18¾ x 11 or 18¾ x 5 or it may be a pyramid with the sections varying in width.

C221—Capacity 1500 Slides—18¾ x 11 x 3¾
For Filing KODACHROME TRANSPARENCIES and 2 x 2" SLIDES

SPECIFICATIONS: All Paragon Tray Drawer Cabinets are made of reinforced steel construction, olive green finish. Interlocking device enables several units to be joined into one. Each sectional unit contains removable drawers with hand grip in front and rear. Interlocking steel base obtainable whenever required. **Constructed according to rigid specifications—not merely adapted.**

Address your orders and inquiries to Dept. P.
Manufactured Exclusively by

PARAGON C. & C. CO., Inc. • 2540 Belmont Ave., New York 58, N. Y.

AND NOW THROUGH

A New Grip on Problems of Industrial Medicine

A Journal Designed For Your Interests

A. M. A. Archives of INDUSTRIAL HYGIENE and OCCUPATIONAL MEDICINE

Edited by men of outstanding reputation in the fields of industrial health and preventive industrial hygiene: Prof. Philip Drinker, Boston, Chief Editor; Robert Kehoe, M.D., Cincinnati; James Sterner, M.D., Rochester, N. Y.; Frank Patty, Detroit, Mich.; Theodore Hatch, Pittsburgh; Frank Princi, M.D., Cincinnati; Fenn E. Poole, M.D., Glendale, Calif.; William A. Sawyer, Rochester, N. Y.

Covering the research and field aspects of industrial hygiene and the clinical and medical aspects of occupational industrial health programs.

Merging the best features of *Occupational Medicine* and *The Journal of Industrial Hygiene and Toxicology.*

Integrated closely with the activities of the Council of Industrial Health of the A. M. A. and the American Industrial Hygiene Association.

A Better and More Useful Journal of Industrial Medicine! Such is the result which the Editorial Board of A. M. A. Archives of INDUSTRIAL HYGIENE and OCCUPATIONAL MEDICINE has achieved by combining parallel publications.

To the thousands of physicians who are directly or indirectly concerned today with medicine in industry, the new journal will bring reports of the continuing and important developments in the field, with original articles covering problems and day to day experiences of physicians in industry; an excellent abstracting service similar to that carried in the *Journal of Industrial Hygiene and Toxicology;* additional foreign journal abstracting; reviews.

Whether servicing industrial firms, attending employees, applying to general practice some of the findings of industrial medicine, or merely *watching* this expanding field, you will want to receive this vital new periodical.

AMERICAN MEDICAL ASSOCIATION
535 N. Dearborn Street, Chicago 10

Enter my subscription to A. M. A. Archives of INDUSTRIAL HYGIENE and OCCUPATIONAL MEDICINE to start *with the next issue.* Per year, $8.00 in U. S. (Canadian, $8.40; Foreign, $9.00).

I enclose check..... Please bill me.....

Name.....

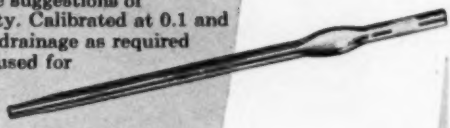
Address.....

for more accurate

Prothrombin Determinations

PIPETTE FOR PROTHROMBIN DETERMINATIONS Designed after the suggestions of Armond J. Quick, M.D., Marquette University. Calibrated at 0.1 and 0.2 ml. Large bore tubing provides the quick drainage as required in prothrombin determinations. May also be used for micro sugar determination. Bulb reduces possibility of accidentally drawing fluid into mouth. Overall length approximately 5½".

No. 67722..... each $1.00 Per dozen..$10.80



WATER BATH Controls water temperature accurately for preparing tissue sections for prothrombin determinations or general incubation work. The heavy duty heating element is thermostatically controlled from room temperature to 60° C. Glass wool insulation.

No. 67075 For 115 v. A.C. Overall sizes 2½" deep x 8½" diameter $45.00

DIFCO / BACTO-THROMBOPLASTIN

A stabilized rabbit brain substance of high thromboplastin potency. May be used in all prothrombin time-procedures using blood and whole or diluted plasma. Approximately 70 tests to an ampule or 420 per standard package.

No. B226 6 ampules.....$4.80

ELECTRIC SECOND TIMER

Readings are direct to 1/10th second. Requires no computations for any interval up to 10,000 seconds. Large, legible numerals ... can be reset to zero from any reading, or successive readings can be totaled. Dust is sealed out by non-breakable plastic window over the direct-drive mechanism.

No. 60925A.....$42.50

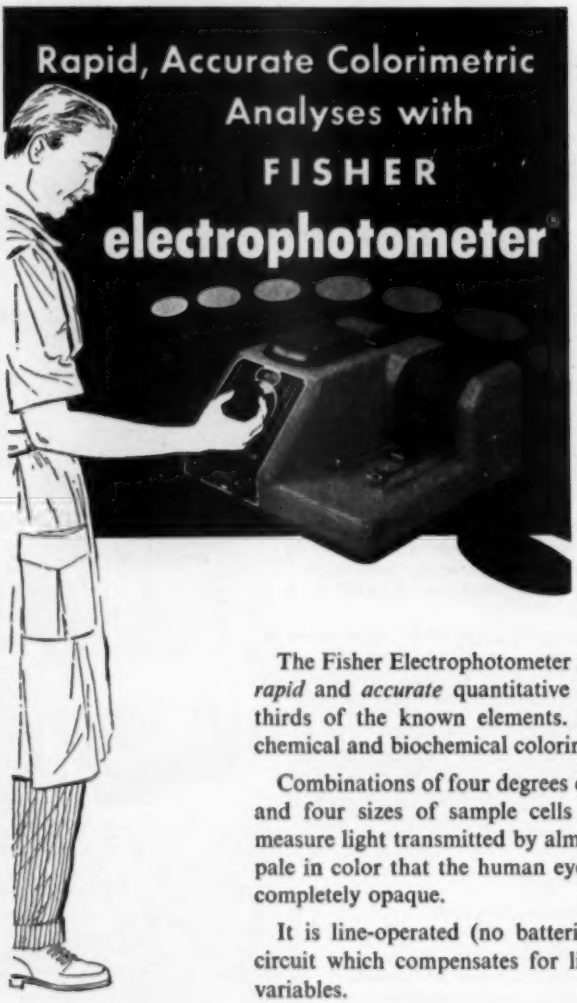
FOOT SWITCH Designed for use with Electric Second Timer (*above*). Especially useful in prothrombin determinations since it leaves both hands free. Push on switch starts or stops Timer. Complete with 3' cord and plug with outlet receptacle; for 115 volts.

No. 68812.....$14.00

Scientific Products Division

AMERICAN HOSPITAL SUPPLY CORPORATION

New York • Chicago • Minneapolis • Atlanta • Washington • Dallas • Los Angeles • San Francisco



The sliding platform holds the cells which contain the blank solution and the sample. After platform is moved into the compartment, the lid is closed. Sliding platform permits positive positioning of the cells.

The dial knob is rotated until the illuminated galvanometer pointer is at the center line. The scale reading is directly related to the concentration of the constituent being measured.

The Fisher Electrophotometer is a colorimetric instrument for the *rapid* and *accurate* quantitative determination of more than two-thirds of the known elements. It is the essential instrument for chemical and biochemical colorimetric analyses.

Combinations of four degrees of light intensity, three color filters, and four sizes of sample cells enable the Electrophotometer to measure light transmitted by almost any solution—whether it be so pale in color that the human eye cannot detect the tint, or almost completely opaque.

It is line-operated (no batteries required) and uses a balanced circuit which compensates for line voltage fluctuations and other variables.

It is extremely versatile and very simple and convenient to operate. A 50-page manual is included with the Electrophotometer which provides easy-to-follow instructions for operation of the instrument, many typical methods, and lists of solutions and apparatus required for each method described.

SEND FOR

ILLUSTRATED BOOKLET FOR COMPLETE INFORMATION ABOUT THIS VERSATILE INSTRUMENT.

Complete stocks of laboratory instruments, apparatus, reagent chemicals, furniture, and supplies at PITTSBURGH, NEW YORK, ST. LOUIS, WASHINGTON, MONTREAL and TORONTO. For more information write: Fisher Scientific Co., 717 Forbes St., Pittsburgh 19, Pennsylvania.

FISHER SCIENTIFIC

America's Largest Manufacturer-Distributor of Laboratory Appliances and Reagent Chemicals

A. M. A.
ARCHIVES OF PATHOLOGY

VOLUME 55 APRIL 1953 NUMBER 4

COPYRIGHT, 1953, BY THE AMERICAN MEDICAL ASSOCIATION

ACID MUCOPOLYSACCHARIDES OF THE AORTA

C. H. BUNTING, M.D.
AND
HENRY BUNTING, M.D.
NEW HAVEN, CONN.

THE PRESENCE in histological sections of the aorta and other blood vessels of a homogeneous ground substance which stains metachromatically with such dyes as polychrome methylene blue, cresylviolet, and toluidine blue has long been noted. Recognition of the mucoid character of this substance and of the fact that it was abundantly present in older and obviously pathological vessels led to the view that it resulted from degeneration of the functional elements of the vessels and thus to the general employment of the term "mucoid degeneration." This term, however, is gradually falling into disuse as the result of an increasing number of demonstrations that this ground substance is constantly present in aortae of all ages, including not only those of the newborn but even those of fetuses of both human beings and animals [1] and is, therefore, a normal constituent of vessel walls. The vessels thus share in the widespread distribution of mucoid substances in normal organs and tissues recently emphasized by Wislocki, Bunting, and Dempsey,[2] who also determined the mucoid material of the aorta to be a mucopolysaccharide of the acid group, consistent with chondroitin sulfate. This identification may account for Krawkow's "aortic amyloid," as suggested by Schultz.[3]

Even casual observation of a properly stained section will convince one of the validity of the uniform descriptions and illustrations of the distribution of this metachromatic substance in the aortic wall, as has been demonstrated by such previous investigators as Jores,[4] Schultz,[3] Wolkoff,[1a] and Ssolowjew.[1b] As commonly described, it is present as a rather diffuse ground substance in the intima, usually with a somewhat increased intensity of staining near the membrana elastica interna.

From the Department of Pathology, Yale University School of Medicine.

This investigation was supported by a research grant from the National Cancer Institute of the National Institutes of Health, United States Public Health Service.

1. (*a*) Wolkoff, K.: Über die Altersveränderungen der Arterien bei Tieren, Virchows Arch. path. Anat. **252**:208, 1924. (*b*) Ssolowjew, A.: Über die Zwischensubstanz der Blutgefässwand, ibid. **241**:1, 1923. (*c*) Orsos, F.: Die Struktur der Aorta ascendens und ihre pathologische Bedeutung, Verhandl. deutsch. path. Gesellsch. **26**:365, 1931.

2. Wislocki, G. B.; Bunting, H., and Dempsey, E. W.: Metachromasia in Mammalian Tissues and Its Relationship to Mucopolysaccharides, Am. J. Anat. **81**:1, 1947.

3. Schultz, A.: Über die Chromotropie des Gefässbindegewebes, Virchows Arch. path. Anat. **239**:415, 1922.

4. Jores, L.: Arterien, Handbuch der speziellen pathologischen Anatomie und Histologie, 1924, Vol. 2, p. 608.

It is still more intensely stained in the media, especially close to the tunica interna, shading off gradually toward the outermost layers of the media, where it may even be unnoticeable. It is closely applied to the elastic lamellae, separating them from other elements, such as muscle cells and fibrocytes. There is general agreement that the mucopolysaccharide increases with age. In fact, Ssolowjew goes so far as to suggest that the increase in thickness of the aortic wall with age is due to it.

The present study, a part of a general consideration of aortic structure at various ages, can add to the picture outlined only a few details of the relation of this mucopolysaccharide to the other elements of the vessel wall which may be important in regard to its function. Presentation of the data may further be justified by the apparent lack of general appreciation of the fact that this ground substance is a constant and normal constituent of the aortic wall from the fetal period throughout the life of even the oldest individuals. It cannot be said that the study deals with "normal aortae." In fact the present study has led to the conclusion that examination of almost numberless specimens would be necessary to establish a histological norm for any age of this active organ, which is subject to so many influencing factors. Among these may be mentioned inherited quality, errors in formation, intrauterine disease, postnatal infections and intoxications, diet, abnormal blood pressure, and mental and physical strain. With regard to such factors, it seems hardly probable that the life of any two individuals would run a parallel course—or that anyone would escape all the factors.

The material for this study consisted of sections of 50 aortae, most of which were taken from the lower thoracic or upper abdominal portion. The tissue was fixed in buffered formalin (pH 7) or in 4% basic lead acetate, and for study of the mucopolysaccharide the sections were stained in toluidine blue.[5] They were cut through areas which grossly were obviously pathological and also through parts which were less so and might be considered to represent the more general state of the vessel wall. The aortae were from persons coming to postmortem examination in the Brady Pathological Laboratory and represented both sexes and all decades from the beginning of the 1st to well into the 10th. To secure this wide distribution, which seemed important, subjects were selected generally because of age rather than because of any special disease or cause of death. As a result, the causes of death varied widely, though it may be said that in the autopsy protocols of a majority of the subjects of advanced years, generalized arteriosclerosis had been entered as an important factor with or without a note of clinical hypertension.

This series includes but one aorta of the fetal period—that of a 5½-month infant who died of a pulmonary infection after two days in the incubator. In the sections cut from this apparently normal vessel, metachromatic ground substance was extensively present adjacent to and on both sides of the membrana elastica interna. It was most marked on the medial side. The greatest development, however, was in the intimal thickening at the mouth of a branch and in the adjacent media. Slight metachromasia was present in other parts of the media.

According to Jores,[4] the aorta of the newborn has no intima, as the endothelium lies directly on the membrana elastica interna. There is, however, scarcely the uni-

5. Bunting, H.: The Distribution of Acid Mucopolysaccharides in Mammalian Tissues as Revealed by Histochemical Methods, Ann. New York Acad. Sc. **52**:977, 1950.

formity of aortic structure in the newborn that this statement would imply, and even where the structure corresponds most closely to Jores' description, a sharply stained specimen shows a definite metachromatic line separating the two membranes (color plate, upper left). In the same section one may note here and there in the intima a few—two, three, or more—flattened fibrocytes in the metachromatic ground substance. Further, in sections passing through the mouth of a branch, as described for the aorta of the premature infant, there is a definite intima with delicate collagen bundles, fine elastic fibers, numerous cells set in a ground substance, and even the beginning of that fibrous crescent which lies at the distal side of such openings. As a further variation of the intima of the newborn, the membrana elastica interna may be doubled for certain distances with no reason for the change obvious. This observation also was true for the aorta of the premature infant.

In the aortic media of the newborn there is an abundance of metachromatic ground substance evenly distributed and, as it were, embracing the elastic lamellae. It follows the picture of the adult aorta in staining more intensely in the inner part adjacent to the membrana elastica interna, but differs from that in the adult in extending throughout the full width of the media. This difference possibly represents a lessened expansion-contraction differential in a thin wall.

With the postnatal growth and development of the individual the aorta grows in length, in diameter and hence in circumference, and in thickness of wall. In the last respect, the growth of an intima is an important factor. This growth is rapid, so that in a few months the wall consists of a cellular and fibrous layer with both collagenous and elastic fibers and a well-marked metachromatic ground substance. As early as the second year there may be a duplication of the membrana elastica interna which adds a heavy elastic lamella to the intima. This stands out in sharp contrast to the fine circular elastic fibers just mentioned. A further addition to the intima is made by the development of the longitudinal musculoelastic layer, which is heavily impregnated with mucopolysaccharide. This is usually present between the membrana elastica interna and its duplication, which lies on its intimal side.

With this development of the intima there is also an increase in thickness of the media. This is apparently not due to an increase in the number of the elastic lamellae, as numerous counts have shown the same number (30 to 35 in the part of the vessel studied, in contrast to the 40 to 60 in the arch) in the aortae of the newborn as in those of adults, with but slight individual variation. There is, however, over several years a variable development of elastic fibers running a somewhat oblique course from one lamella to the next. One must also assume a growth of smooth-muscle cells to supply the increased circumferential expansion of the vessel. Further, the increase in polysaccharide in the media with growth may add to its thickness to a certain extent, in accordance with the opinion of Ssolowjew already noted.

The general statement that the mucopolysaccharide in the media increases with age apparently needs certain qualifications. This ground substance, beginning to increase immediately after birth, continues to develop during childhood and youth and, as shown definitely in the series of cases forming the basis of this report, reaches a maximum amount and the most uniform distribution in the third and fourth decades (color plate, upper right). It then separates the elastic lamellae from

all other elements. In accordance with the usual description, the metachromasia is most intense in the inner third of the media, paling out in the middle third and being usually unobservable in most of the outer third.

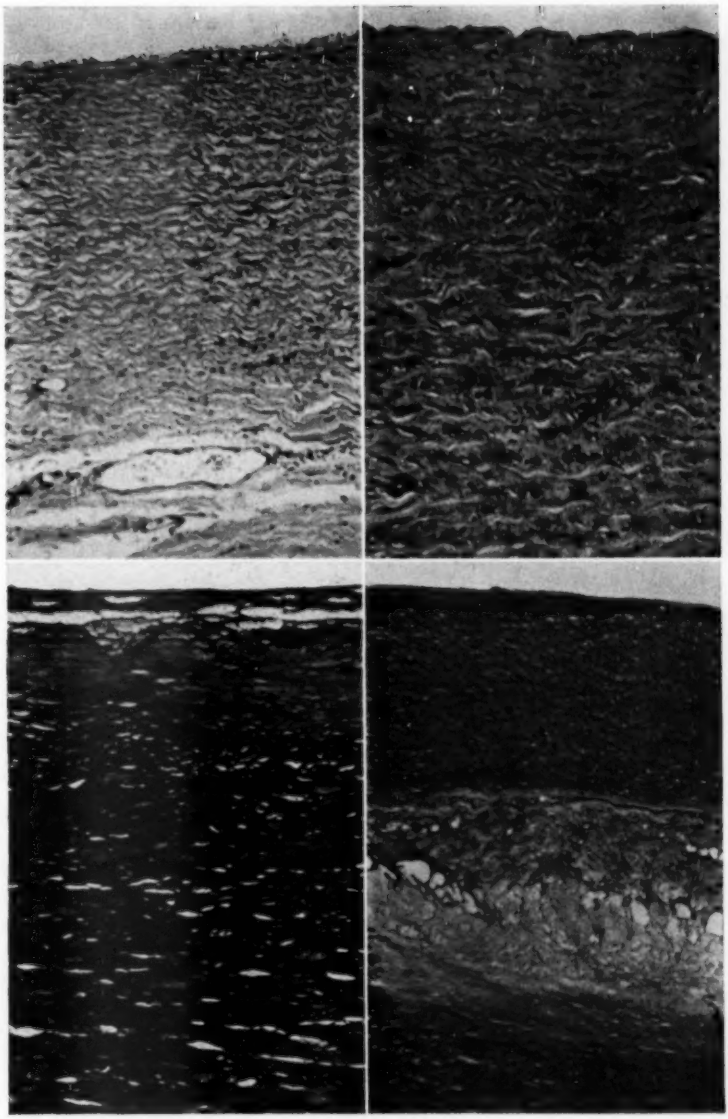
There is no sharp dividing line between the aortae of this period and those of the immediately succeeding decades. Yet as the years increase, with the manifestations of continued wear and tear in degenerations of elastic fibers and muscle and an increase in collagenous fibers, there is a definite decrease in metachromatic ground substance, until in the inelastic vessels of extreme age this substance may be almost entirely lacking over a considerable part of the circumference (color plate, lower left). This lack is especially striking in the atrophic media beneath large nodular thickenings of the intima. In the older vessels there is also a greater irregularity in the distribution of ground substance. Accumulations of varied extent occur where there is obvious destruction of elastic tissue or of elastic tissue and muscle. No marked increase is found where muscle alone has disappeared. Under that circumstance various lamellae simply collapse upon each other without any definite increase of acid mucopolysaccharide. This fact has recently been commented on by Gore and Seiwert.[6] While these "lakes" of ground substance may occur at any age, they are more numerous in the older vessels. They vary in size up to the so-called "mucous cysts" found in the "idiopathic medial necrosis"[7] that may lead to dissecting aneurysm. Similar extensive accumulations may be found in the areas of medial destruction in syphilis of the aorta and likewise in the areas of destruction of unknown causation frequently seen about the vasa vasorum.

Acid mucopolysaccharide is present as a ground substance in the intima throughout life, yet owing to the greatly varied pathological processes which occur in this layer, with distortive and destructive effects, distinct trends of increase and decrease of the substance cannot be outlined as they have been for the media. In the intima of the adult vessel in areas without nodule formation but with cellular and fibrous increase in thickness, there is a fairly uniform presence of metachromatic ground substance throughout its width. The material is so widespread that no definite relationship to the fine elastic fibers can be determined, but the heavy duplication band or bands from the membrana elastica interna are as closely surrounded as are the medial lamellae. The ground substance varies in intensity of staining, as it not infrequently is more sharply stained close to the endothelium and is always deeply stained toward the membrana elastica interna. This variation is especially marked if a longitudinal musculoelastic layer is present. The picture may be varied in numerous ways by the occurrence of pathological changes. The ground substance may be displaced by extensive accumulations of lipoid-containing phagocytes and free lipoid droplets. There may be a marked dilution of the substance, apparently by serous imbibition, as indicated by paleness of staining; on the other hand, intensely staining streaks of various widths may extend along certain fibrous bundles.

The most striking development of metachromatic material in the intima is found in the cellular and fibrous material of the nodules of sclerosis (color plate, lower

6. Gore, I., and Seiwert, V. J.: Dissecting Aneurysm of the Aorta, A. M. A. Arch. Path. **53**:121, 1952.

7. Erdheim, J.: Medionecrosis aortae idiopathica, Virchows Arch. path. Anat. **273**:454, 1929.



Upper left, aorta of stillborn full-term infant. Entire thickness of aorta is included; intima is at top of photomicrograph. Definite line of metachromatic staining separates endothelium from elastic intima. Toluidine blue; × 118. Upper right, aorta of man aged 23. Intima and much of media are included in photomicrograph. Metachromatic ground substance is abundant. Toluidine blue; × 118. Lower left, aorta of woman aged 83. Little metachromatic ground substance is present in intima and media. Toluidine blue; × 118. Lower right, atheromatous nodule from aorta of woman aged 77. Cellular collagenous tissue contains abundant metachromatic ground substance, while atheromatous nodule has little. Portion of media, included at bottom of the photomicrograph contains none. Toluidine blue; × 118.

right) and in the similar intimal reaction in syphilis. In these areas the fibrocytes are sharply outlined by metachromatic material which extends from them in streaks between dense collagenous bundles or which in looser material seems to impregnate the bundles, thus establishing a definite relation to collagenous fibers rather than to elastic tissue. As noted by Schultz, where this tissue becomes cell-poor and hyaline, no metachromasia is found. Metachromasia is also lacking in the necrotic disintegrating material which forms the center of atheromatous nodules.

Metachromasia is commonly not found in the rather loose-appearing adventitia of the aorta in the area studied, although elastic fibers are not lacking in it. An exception to this rule, however, is offered by the elastic, tissue-rich adventitia at the angle between the vessel and an emerging branch. Here it may be well marked. It has been noted also that in the carotid arteries it may extend not only entirely through the media but even into the inner part of the dense adventitia.

The accepted opinion that the mucopolysaccharide of an unfixed aorta had the same physical characteristics as mucoid substances elsewhere in the body was made a certainty by Erdheim's [8] puncture of a "mucous cyst" in a case of dissecting aneurysm. In frozen sections of a formalin-fixed aorta this ground substance appears as a structureless homogeneous translucent material. In sections prepared by the usual paraffin technique the material is still translucent but is extensively vacuolated. As the vacuoles are demonstrably not lipoid in character, there is every reason to accept Schultz's explanation that they are due to dehydration by the alcohol through which the tissue has passed. The vacuoles vary much in size and in number in any area, and the variations seem to follow fairly closely changes in intensity of staining. In the very deeply stained streaks, vacuoles are few and so small as to be inconspicuous. In the heavily stained material close to the membrana elastica interna, they are small but numerous. In the faintly stained areas they are large and numerous and frequently outlined by an intensely staining line. Dehydration as a causative factor for these vacuoles being accepted, the variations would strongly imply that the variation in staining of the ground substance is due to a variation in concentration of the acid mucopolysaccharide in the tissue fluids.

In the "mucous cysts" of one case of dissecting aneurysm in this series there was no evidence that one was dealing with myxomatous tissue, as claimed by Gore and Seiwert [6] and Gore.[9] The mucoid substance was cell-free and the only fibrils present were detached, degenerating elastic fragments. No attempt has been made to check the findings of Rinehart and Greenberg [10] that under the electron microscope the mucopolysaccharide is rich in "extremely delicate fibrils."

The nature of the acid mucopolysaccharides as revealed by the metachromatic staining with toluidine blue has been sought by use of hyaluronidases. The enzyme of testis origin is known to act upon mucopolysaccharides that contain sulfate as well as upon those that do not, while that derived from streptococci acts only upon

8. Erdheim, J.: Medionecrosis aortae idiopathica cystica, Virchows Arch. path. Anat. **276**:189, 1930.

9. Gore, I.: Pathogenesis of Dissecting Aneurysm of the Aorta, A. M. A. Arch. Path. **53**:142, 1952.

10. Rinehart, J. F., and Greenberg, L. D.: Pathogenesis of Experimental Arteriosclerosis in Pyridoxine Deficiency, with Notes on Similarities to Human Arteriosclerosis, A. M. A. Arch. Path. **51**:12, 1951.

the latter group.[11] This observation has been made use of in histochemical differentiation of acid mucopolysaccharides as found in tissues. The metachromatic staining of hyaline cartilage, known to contain chondroitin sulfate, is prevented by previous treatment with testis hyaluronidase but not altered by streptococcal enzyme. The metachromatic staining of synovial fluid, known to contain hyaluronic acid only, is eliminated by treatment with streptococcal as well as by testis enzyme. In the present test of the aortic metachromatic substance the enzyme preparations were made up at a concentration of 15 turbidity reducing units per milliliter in 0.3% NaCl at a pH of 6.0 to 6.3.[5] Incubation was carried out for 18 hours at 37 C. Control preparations were similarly incubated in 0.3% NaCl, as it had been previously determined that this procedure was no different in effect from incubation in boiled enzyme preparations.

The metachromatic staining of the aorta did not occur subsequent to exposure to the testicular enzyme but was not materially altered by exposure to streptococcal enzyme or to the control saline solution. Mast-cell granules were not affected in their subsequent staining by either enzyme.

The demonstration that the mucopolysaccharide was not a product of degeneration but a physiological component of vessel walls immediately raised the questions of its origin and of its function. Neither question has had a full and satisfactory answer to date. The fact that the mucopolysaccharide has been found only in cellular areas and often sharply outlines fibroblasts in those areas has led one of us [5] to the opinion that these cells are responsible for it. The present study would tend to strengthen that opinion. Gersh,[12] in discussing the ground substance of connective tissues, states that "connective tissue cells not necessarily fibroblasts may produce the ground substance and are also involved in controlling its state of polymerization" but later admits that it is "probably the fibroblast" that gives rise to it. Ham [13] is also of the opinion that the ground substance is a product of the connective-tissue cells and suggests also that the cells have some of the properties of chondrocytes, as under certain pathological conditions they form cartilage and later bone. A suggestion that the mast cells might be the origin (Sylvén,[14] Asboe-Hansen [15]) finds no support in the aorta, for, while the mast cells may be numerous in the adventitia, mucoid material is lacking there, and in the inner media, where it is abundant, mast cells are lacking.

Suggestions as to function have been more varied. The mucopolysaccharide is generally accepted as a ground substance which not only is widespread in normal areas but also fills in pathological defects in the aortic wall. Its close association

11. Meyer, K.; Chaffee, E.; Hobby, G. L., and Dawson, M. H.: Hyaluronidases of Bacterial and Animal Origin, J. Exper. Med. **73**:309, 1941.

12. Gersh, I.: Some Functional Considerations of Ground Substance of Connective Tissues, in Conference on Connective Tissues, Transactions of the Second Conference, edited by C. Ragan, May 24-25, New York, Josiah Macy, Jr. Foundation, 1951, p. 11.

13. Ham, A. W.: Histology, Philadelphia, J. B. Lippincott Company, 1950.

14. Sylvén, B.: Über das Vorkommen von hochmolekularen Esterschwefelsäueren im Granulationsgewebe und bei der Epithelregeneration, Acta chir. scandinav. (Supp. 66) **86**:1, 1941. Ester Sulfuric Acids of High Molecular Weight and Mast Cells in Mesenchymal Tumors, Acta radiol., Supp. 59, pp. 1-99, 1945.

15. Hansen, G. A.: The Origin of Synovial Mucin: Ehrlich's Mast Cell, a Secretory Element of the Connective Tissue, Ann. Dermat. Dis. **9**:149, 1950.

with elastic tissue has led to speculation as to a possible nutritive function in respect to that tissue. Schultz [3] expressed the opinion that it furnishes materials for the building up of elastin and suggested that chondroitin might be among them. Wolkoff [1a] also was of the opinion that it plays an important part in the nourishment of the embedded elements. Ssolowjew,[1b] noting that it is present in tissues devoid of blood vessels, suggested that it may transmit nourishment by diffusion. Wislocki, Bunting, and Dempsey [2] have recently emphasized that in the wide distribution of such metachromatic substances they are found chiefly in nonvascular areas or in poorly vascularized tissue, such as the blood vessels, and to this observation the only exceptions are the papillae of the skin and the mucous membranes. According to Rinehart and Greenberg,[10] the mucoid material is probably "a mother substance which normally acts as an effective cement substance and from which collagen and elastic fibers are maintained and formed."

Orsos [1c] expressed the opinion that this mucoid substance furnishes an embedding material for the other elements of the aorta and as a result of its physical character permits a frictionless rhythmic shifting of those elements in relation to each other. This possible function has recently been emphasized.[16] The present study adds support to the theory that this is at least one of the functions of this ground substance even though it may not be the most important. As has been noted earlier, the substance is present in greatest quantity and is most evenly distributed in what is physically the most active period of life but is diminished in the late senile inelastic state of the vessel. Further, it is most extensively developed in the inner part of the vessel wall, where one must picture during expansion and contraction due to the pulse wave a shifting of elements in relation to each other greater than that in the outermost layers of the media.[17] The acceptance of this as one function would not rule out the nutritive value of the substance, which has, however, thus far not been definitely proved.

The question whether accumulations of mucopolysaccharide in the vessel wall might lead to harmful effects was raised by Erdheim.[8] In his discussion of dissecting aneurysms of the aorta he expresses himself as tempted to make the mucoid degeneration answerable for the wide destruction of the media in the region of the aneurysm. It is at present generally accepted that the reverse is true, that the primary destruction of the media is followed by an accumulation of the mucoid substance. According to Gore and Seiwert,[6] the mucoid substance is propelled to this point from other regions as a result of arterial tension to which the wall is subject. According to Gore,[9] under this pressure the wall may "even dissect along lines of weakness resulting from the loss of anastomotic fibers."

While this study has concerned the aorta chiefly, it may be said that in arteries of the elastic type the same ground substance is present and has the same general distribution except that it is present throughout the thickness of the media and may,

16. Wislocki, Bunting, and Dempsey.[2] Bunting.[5]

17. Acceptance of the conclusions of Reynolds and associates (Reynolds, S. R. M.; Light, F. W.; Ardran, G. M., and Prichard, M. M. L.: The Qualitative Nature of Pulsatile Flow in Umbilical Blood Vessels, with Observations on Flow in the Aorta, Bull. Johns Hopkins Hosp. **91**:83, 1952) that there is "no detectable alteration in the calibre of large arteries such as the umbilical artery and the descending aorta with the pulse pressure" would require another explanation of this difference.

as mentioned for the carotid, extend into the adventitia. In arteries of the muscular type the ground substance is commonly limited to the region of the membrana elastica interna, where it is present on both the intimal and the medial sides.

SUMMARY

In summation it may be said that the acid mucopolysaccharide under discussion is a normal constituent of the aortic wall and is present to some degree from the fetal period throughout the life of the individual. In the media it increases in amount into the fourth decade, after which, with lessened elasticity and increased fibrosis, it gradually diminishes. In the intima, on the other hand, its maximum development appears to be in the dense fibrous pathological lesions of later life until these become acellular and hyaline.

Functionally, it serves definitely as a ground substance and as such fills pathological defects. A further function appears to be that, owing to its physical characteristics, it is a medium in which elements which shift in relation to each other in the expansion and contraction of the vessel may have free movement. While nutritive functions are probable, they are yet to be definitely demonstrated.

Origin of this material from fibrocytes appears probable but as yet is not definitely proved.

EFFECTS OF HIGH INTENSITY RADIANT ENERGY ON SKIN

I. Type of Injury and Its Relation to Energy Delivery Rate

G. E. SHELINE, Ph.D., M.D.

E. L. ALPEN, Ph.D.

P. R. KUHL, A.B.

AND

A. J. AHOKAS, A.B.

SAN FRANCISCO

EXPERIMENTAL work on thermal injury in the past has dealt largely with the nature of the systemic effects following severe burns. Recently such groups as Henriques and Moritz at Harvard;[1] Pearse, Payne, and Hogg at Rochester,[2] and Leach, Peters, and Rossiter at Oxford [3] have undertaken studies of the nature of the local damage following thermal trauma. An understanding of the nature of the local injury, both immediate and delayed, would be useful in anticipating the cause and nature of the systemic changes and in determining proper management of the burned patient.

In the present studies burn lesions were produced by penetrating radiant energy delivered under carefully controlled conditions. The energy intensity available was great enough so that short exposures, comparable to those of many "flash burns," were capable of effecting burns of considerable severity. Observations were made on the gross and microscopic pathology of the lesions for investigation of the relation between these changes and the energy delivery rate. The data obtained are

---

From the Division of Biological and Medical Sciences, United States Naval Radiological Defense Laboratory.

This work was made possible, in part, by the Armed Forces Special Weapons Project and by the Bureau of Medicine and Surgery, Department of the Navy.

This article has been released for publication by the Division of Publications of the Bureau of Medicine and Surgery of the United States Navy. The opinions or assertions contained herein are the private ones of the authors and are not to be construed as official or reflecting the views of the Navy Department or the naval service at large.

1. (*a*) Henriques, F. C., Jr., and Moritz, A. R.: Studies of Thermal Injury: I. The Conduction of Heat to and Through the Skin and the Temperatures Attained Therein: A Theoretical and an Experimental Investigation, Am. J. Path. **23**:531, 1947. (*b*) Moritz, A. R., and Henriques, F. C., Jr.: Studies of Thermal Injury: II. The Relative Importance of Time and Surface Temperature in the Causation of Cutaneous Burns, ibid. **23**:695, 1947.

2. Pearse, H. E.; Payne, J. T., and Hogg, L.: The Experimental Study of Flash-Burns, Ann. Surg. **130**:774, 1949.

3. Leach, E. H.; Peters, R. A., and Rossiter, R. J.: Experimental Thermal Burns, Especially the Moderate Temperature Burn, Quart. J. Exper. Physiol. **32**:67, 1943.

correlated with those previously reported in the literature for burns produced by nonradiant thermal sources, i. e., contact with hot liquids, solids, or flame. It is thought that these studies will aid in the extrapolation of the information already available for burns otherwise produced to that for burns produced by radiant energy.

METHODS

A 36-in. standard navy searchlight fitted with a modified Mole-Richardson arc mechanism was used as the thermal source. The arc was operated at 76 volts and 170 amp. DC with 16 mm. National Carbon Co. "super high intensity searchlight carbons." The arc crater was at the focal point (focus *a*, Fig. 1) of a paraboloidal reflector which collimated the light. The main portion of the beam passed through a partially reflecting plane glass [4] surface and was refocused by a second paraboloidal reflector. The reflected portion of the beam was focused by a third paraboloidal reflector. The main beam was used as the thermal source for the burns, while the reflected portion permitted monitoring of the beam energy during exposure. For

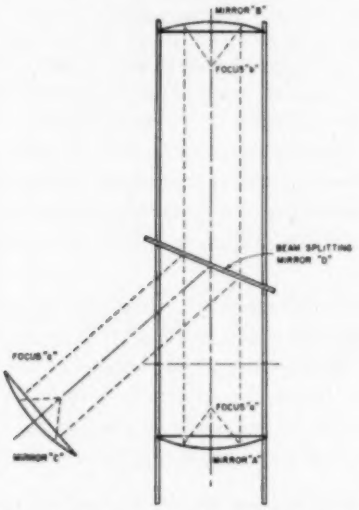


Fig. 1.—Equipment used for thermal radiation burns. *A*, *B*, and *C* are paraboloidal mirrors. *A* and *B* have front aluminized surfaces. Carbon arc crater is at focal point *a*, object to be burned at focal point *b*, and monitoring calorimeter at focal point *c*. Glass sheet, *D*, reflects a portion of entire collimated beam to mirror *C*.

calibration a black body calorimeter was placed at each of the two foci of the divided collimated beam: the first calorimeter, at focus *b*, measured the energy in the main beam; the second, or monitoring, calorimeter, at focus *c*, measured the portion reflected from the beam-splitting mirror. In this way a ratio between the two portions of the beam and a direct measurement of the energy delivered to the burn area were obtained. Data obtained from the monitoring calorimeter permitted a calculation of the energy delivered by the main beam when an animal holder was substituted for the first calorimeter. Under these operating conditions the maximum intensity which could be delivered to the receiver was 85 calories per square centimeter per second. The spectral distribution of the energy incident on the receiver was essentially that of a black body radiator at a temperature of 5,000 to 6,000 K. with the exception of the attenua-

4. Libbey-Owens-Ford "color clear" glass.

tion produced by the glass searchlight window and the plane glass beam splitter. This attenuation was such that no energy remained in the final focused beam below 3,100 A. or above 22,000 A.

The searchlight was mounted in such fashion that it was rotatable about a vertical axis. Rotation was accomplished by means of an electric motor, while reduction gears allowed a wide range of closely controlled rotational speeds. In this manner a reproducible, nearly triangular energy pulse was delivered to the receiver. The time of exposure, which is defined as the time required to deliver the total energy, assuming it was all delivered at the maximum rate of the pulse, was varied by changing the rotational speed of the searchlight.

Change of intensity was accomplished by placing different combinations of wire screen, expanded metal, or drilled metal sheets in the collimated beam between the searchlight window and the beam-splitting mirror (Fig. 1). Thus the beam intensity could be varied in discrete steps from a fraction of 1% of full beam intensity to full beam intensity. Throughout this paper

Fig. 2.—Animal holder, supporting mill table, and paraboloidal mirror *B* of Figure 1. Holder is turned sideways so that aperture and steps of constant-temperature, aperture-defining copper plate can be seen. Adjustable table and protective back plate of holder are visible.

reference to "energy applied" means the calorimetrically measured energy, expressed in gram calories, with no correction made for reflection or scattering losses.

The animal holder (Fig. 2) shielded the rat from all radiation originating from the carbon arc except that passing through a circular aperture 0.9 cm. in diameter placed at the focus of the main beam. This aperture was defined by a copper plate maintained at 35 C. by water from a thermostat-controlled bath circulating through its hollow interior. The face of this plate was machined in a series of steps with dimensions such that light originating from the arc and reflecting off a step could not enter the aperture. The inner step, which defined the aperture, was 1/64 in. thick and was covered on the inside by a plastic film. An asbestos liner prevented contact between the animal's skin and the metal holder.

Female Sprague-Dawley rats from the laboratory breeding colony were used. The animals were sexed at birth, segregated, and maintained on a standard diet of purina laboratory chow.*

At 50 to 60 days after birth the animals were depilated. After close clipping during light pentobarbital (nembutal®) anesthesia, a thick paste of a commercial strontium sulfide depilatory was applied to the right and left flank and hip areas and allowed to remain four to five minutes. The paste was washed off in a stream of cool water, a rinse of 0.1% acetic acid applied, and a final rinse of cool water given. Depilation was followed immediately and two and four days later by application of wool fat. The animals were burned on the seventh or eighth day after depilation. In a study made on the effect of this depilation technique it was found that at seven to eight days the skin was essentially normal and regenerating hair shafts had just reached the epidermal surface.[5]

For exposure, the rats were anesthetized by subcutaneously administered pentobarbital (nembutal®) solution (40 mg. per kilogram) and held lightly in position against the aperture of the animal exposure holder. A burn was administered to the skin over the left and right hip. Immediately after burning, the physical appearance and the size of the lesion were recorded and a 35-mm. kodachrome® photograph made. Twenty-four hours later changes in physical appearance were recorded and the lesions again photographed.

For histological study the burn from the left hip was excised, mounted on a wooden tongue blade with Michel wound clips, and fixed in Bouin's solution. The specimens were imbedded, sectioned, and stained with hematoxylin and eosin. Unless otherwise noted, biopsies were all done 24 hours after the burn exposure. Specimens from the equivalent skin area of nonburned animals were used as normal controls. All histological conclusions are based on data taken by two observers, each of whom examined every slide at least three times on different days.

RESULTS

The initial burns demonstrated that a sufficient increase in the energy dose incident on the animal's skin resulted in a burn of different gross appearance. Furthermore, when a particular dose was given to several animals the burns were very much alike, and lesions burned on animals on different days were comparable. For the same energy treatment a high degree of uniformity of tissue damage was also apparent in the histological appearance of the lesions. Over a dose range varying from that necessary to produce a barely perceptible tissue change to that required for complete destruction of the entire skin thickness the burn lesions could be classified on the basis of gross and histological appearance. Of the series of burns made with varying energy doses, 2 to 67 calories per square centimeter, and with varying times of delivery, 0.1 to 8 sec., the series of burns for the 0.4-sec. exposure was arbitrarily selected as the common standard against which other burns could be compared. Accordingly a severity spectrum was set up for the 0.4-sec. burns based on the pathology of the 24-hour postburn specimens. This spectrum is outlined in Tables 1*A* and 1*B*.

Gross Observations.—Since the 0.4-sec. exposure series was used as the comparison reference, the pathological changes of this series will be given in some detail. Immediately after burning, the minimum change detectable was an erythema which developed within two to five minutes and faded within the first half-hour; 3.5 to 4.0 cal./cm.^{2} was required to produce this lesion. A more lasting erythema was not seen in these studies. When the incident energy was increased to 4.5 cal./cm.,^{2} a pinkish-white lesion was found. As the amount of energy was increased over that necessary to produce this lesion, the pinkish cast disappeared,

5. Kuhl, P. R.; Sheline, G. E., and Alpen, E. L.: Strontium Sulfide Depilation of Rat Skin and the Effect of Lanolin on Depilated Skin, U. S. Naval Radiological Defense Laboratory Report USNRDL-345, 1952, p. 1.

and the burned area became more chalky-white. In the region of 7 to 8 cal./cm.^{2} there was a light-gray cast to a predominantly white lesion. This gray became more pronounced as the energy delivered was increased and reached a maximum at about 12 to 15 cal./cm.^{2} In the energy range from 12 to 15 cal./cm.^{2} to about 25 cal./cm.,^{2} multiple tiny, thin-walled, empty vesicles appeared on the

TABLE 1*A*.—*Summary of Gross and Microscopic Appearance of 0.4 Sec. Burns*

Energy, Cal./Cm.^{2}	Gross Description		Microscopic Appearance of Epidermis at 24 Hr.	
	Immediate	24 Hr.		
3.0-3.2	No change	No change	No change seen	(0)*
3.5-4.0	Transient erythema	White	Nuclei and cytoplasm pale; cells swollen and eosinophilic	(1)
4.5-5.0	White	Serous brown crust	Absent†	(1)
6.0-9.5	Grading from white through light gray with increasing energy		Thinned and structureless except for pyknotic, basophilic nuclei	(2)
12-15	Dark gray and scaly		Multiple exploded blisters	(3)
20-21	Dark gray and scaly		Thin structureless fragments	(3)
22-24	Dark gray and scaly		Absent‡	(4)

* Numbers in parentheses are the arbitrary severity scores assigned to each description.
† Replaced by a serous brown crust.
‡ Burned off.

TABLE 1*B*.—*Summary of Gross and Microscopic Appearance of 0.4-Sec. Burns*

Energy, Cal./Cm.^{2}	Dermis at 24 Hr.			
	Vascular Supply	Collagen	Leucocyte Infiltration	
3.0-3.2	Increased at surface	No change seen	None	(1)*
3.5-4.0	Increased in upper ⅓	No change seen	Frequent, scattered	(1)
4.5-5.0	Necrotic Structureless Mass Replaces Upper Third			
	Moderate increase in remaining dermis	Minimal coagulation	Massive	(2)
5.3	No increase over normal	Moderate coagulation with some structure lost	Few	(3)
5.9-6.1	Vessels not seen in upper ⅓	Same as 5.3	None	(3)
6.5-7.5	None seen	Moderate coagulation with structure lost and basophilia	None	(4)
8.0-9.0	None seen	Complete coagulation in upper ⅓ to ⅖; collagen appears to be without structure	None	(4)
12-15	None seen	Complete coagulation with increasing basophilia; foamy on bottom ⅓	None	(5)
20-21	None seen	Marked thinning and basophilia; lower ⅓ foamy	None	(5)

* Numbers in parentheses are the arbitrary severity scores assigned to each description.

surface of the gray lesion. For convenience these burns are referred to as "scale" burns. At energy deliveries greater than 25 cal./cm.^{2} frank charring of the skin occurred.

Twenty-four hours after burning, animals exposed to 3.5 to 4.0 cal./cm.,^{2} i. e., those exhibiting the early transient erythema, had a white lesion comparable to the white of the 4.5 cal./cm.,^{2} zero time observation. Those having white burns (4.8 to 6.1 cal./cm.^{2}) at zero time had areas of serous encrustation overlying the white

lesion. With energies around 4.5 cal./cm.² this serous encrustation replaced completely, or nearly completely, the white coagulation. The area of encrustation diminished at higher energies and in the 5.5 to 6.0 cal./cm.² range was found only at the periphery of the lesion. Burns produced by 7 cal./cm.² or above were essentially the same at 24 hours as immediately after burning.

Burn series produced in exposure times from 0.1 to 8.0 sec. showed spectra of changes similar to that seen for 0.4 sec. Certain features of the zero time spectra are indicated in Figure 3. A burn plotted as "white" or "gray" was predominantly

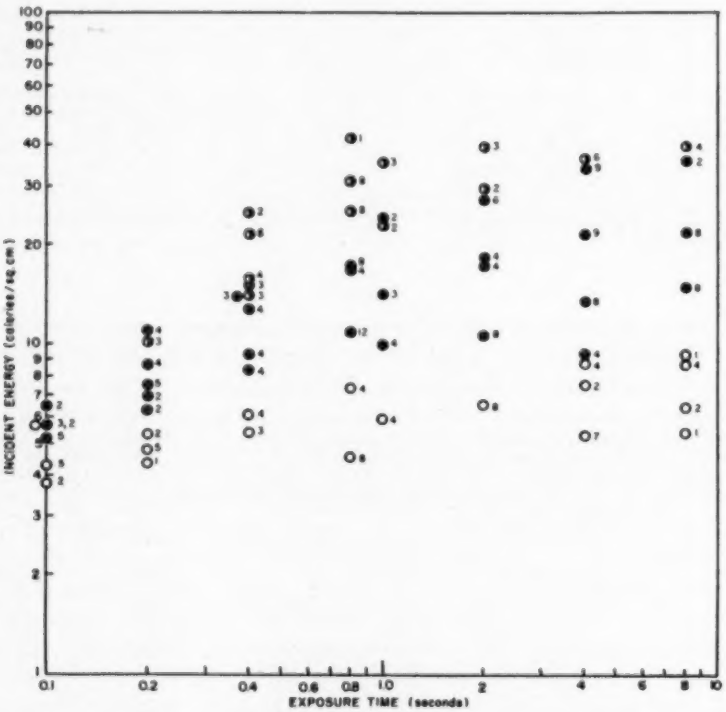


Fig. 3.—Total energy delivered to rat skin plotted against exposure time for three types of burn. Symbols (empty circle, solid circle, and half-solid circle) indicate that burn surface was white, gray, or scale, respectively. Number next to each symbol shows number of observations represented by symbol.

the color plotted; however, some lesions in the transition zones contained areas of both colors. In this chart all variations in degree of grayness are classed together. The number of times a particular observation was made is shown next to the plotted symbol. The lowest energy at which a white lesion was found was 3.7 cal./cm.² at 0.1 sec., 5.3 at 0.4 sec., 5.9 at 1.0 sec., and 5.5 at 8 sec. The minimum incident energy in cal./cm.² necessary to produce a gray color was 6.2, 8.4, 9.9, and 15.5 and that necessary to produce a scaly surface was 10.0, 13.5, 24, and 40, at 0.2,

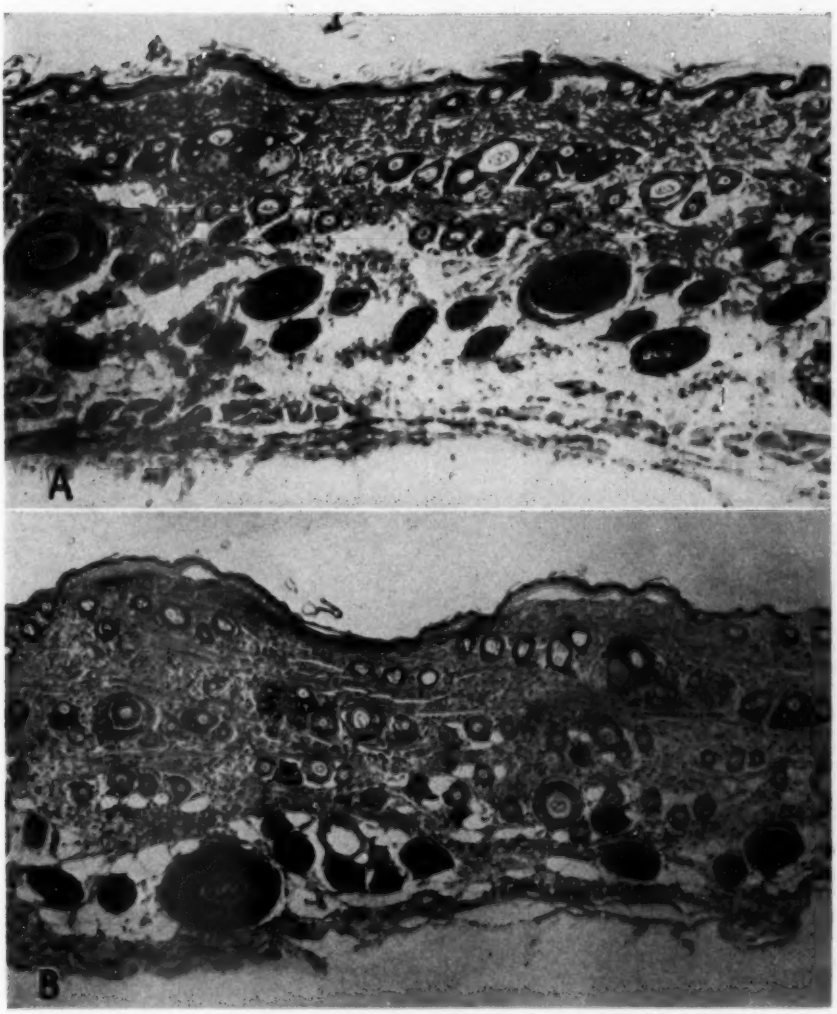


Fig. 4.—Figures 4, 5, and 6 show effect on rat skin of various amounts of radiant thermal energy. All exposure times were 0.4 sec. Hematoxylin and eosin stain; × 100. *A*, section showing epidermis and dermis of depilated nonburned rat skin. *B*, appearance 24 hours after application of 4.0 cal./cm.^{2} Epidermis is pale-staining and cells are swollen. There is an increase in vascularity in superficial dermis, and polymorphonuclear leucocytes are scattered through dermis. A severity score of 1 was assigned to epidermis and dermis of burn lesions with this microscopic appearance.

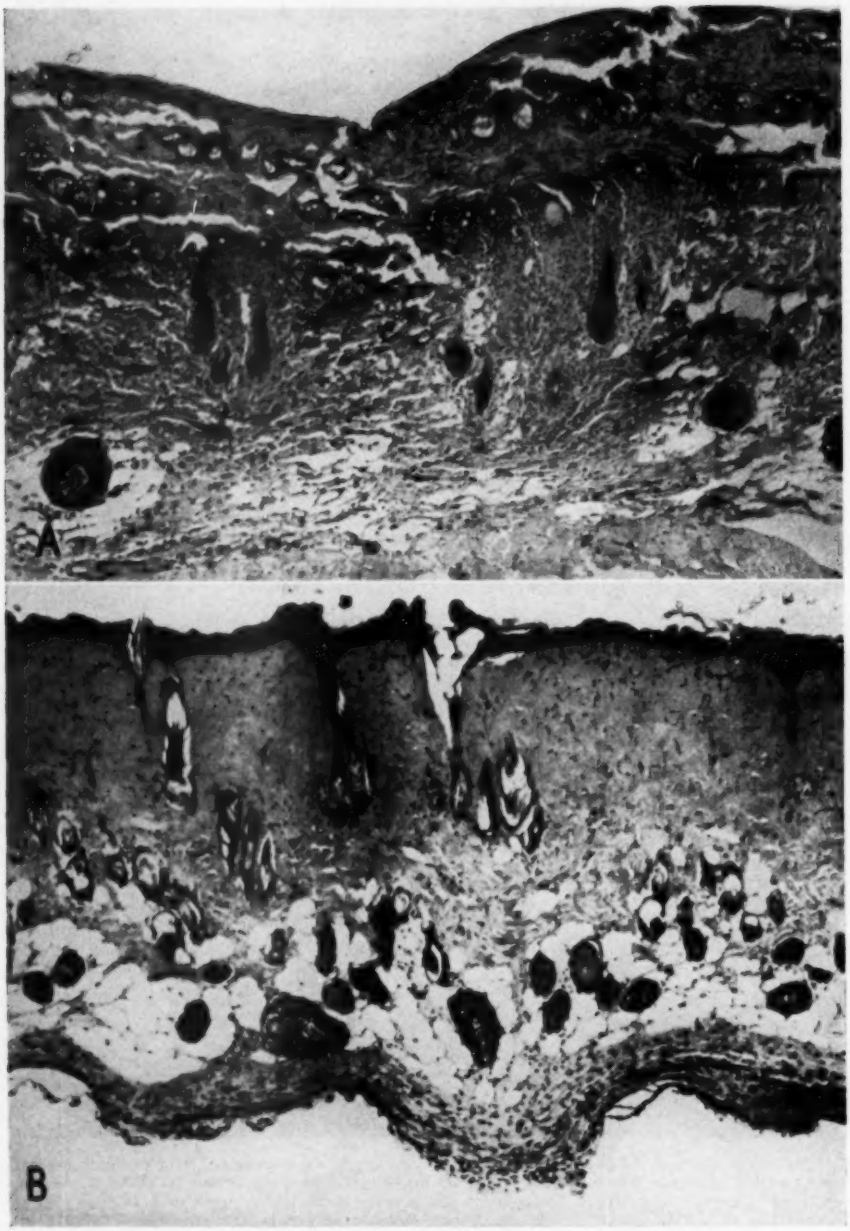


Fig. 5.—*A*, 24 hours after application of 4.8 cal./cm.^{2} Epidermis easily rubs free from dermis at this energy level and in this figure is absent. Upper dermis is replaced by an eosinophilic structureless mass. Massive infiltration of polymorphonuclear leucocytes and increased vascularity are seen in deeper dermis. Severity scores of 1 and 2 were assigned to epidermis and dermis, respectively. *B*, 24 hours after application of 9.1 cal./cm.^{2} Epidermis shows increased basophilia and except for pyknotic nuclei is without visible cellular structure. Complete coagulation is seen in upper half of dermis, while remainder shows less complete coagulation. No patent vessels are seen. Severity scores of 2 and 4 were assigned to epidermis and dermis, respectively.

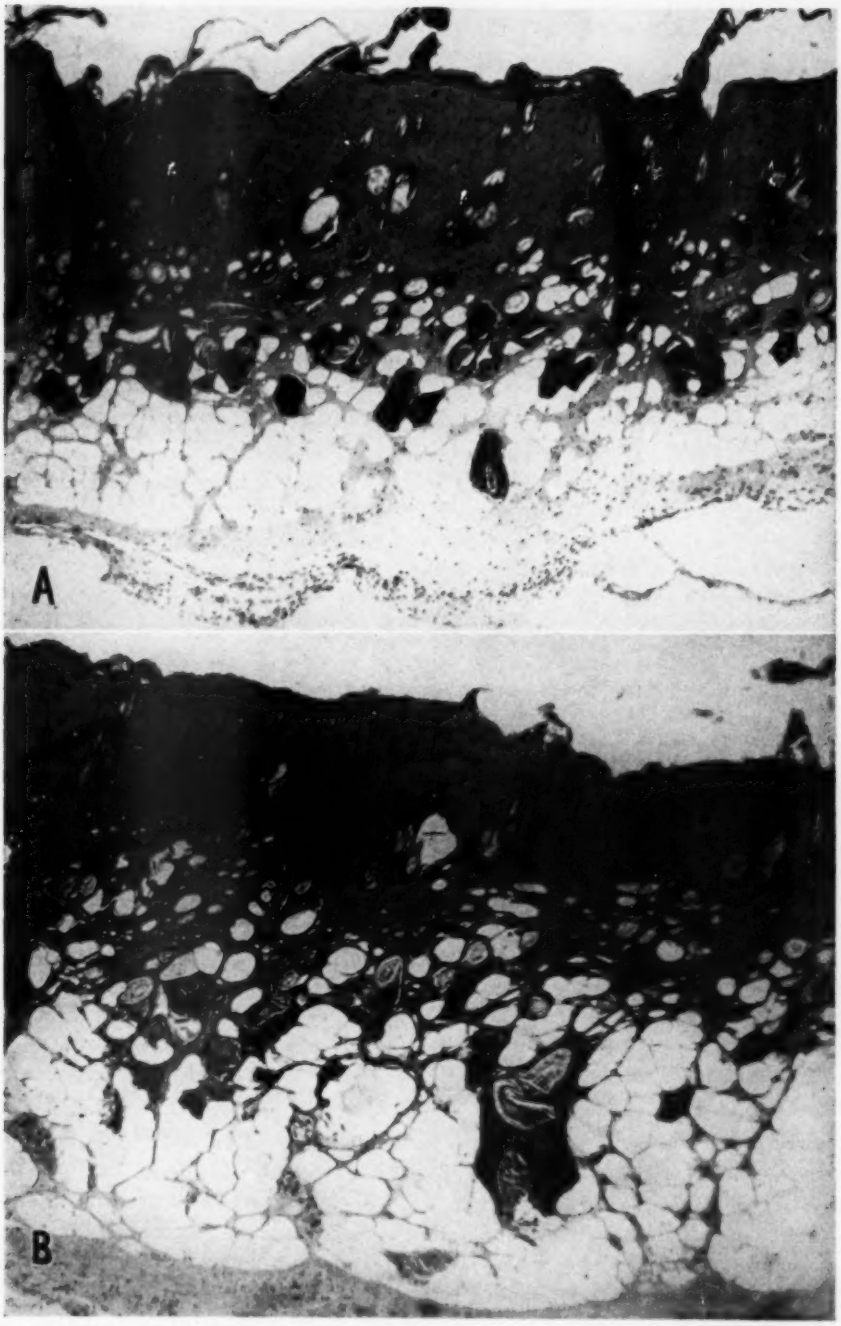


Fig. 6.—*A*, 24 hours after application of 12.5 cal./cm.^{2} Epidermis is thin and fragmented and has appearance of many empty exploded blisters. Dermis is basophilic and completely coagulated, with empty bubble-like spaces in base. Severity scores of 3 and 5 were assigned to epidermis and dermis, respectively. *B*, 24 hours after application of 23.9 cal./cm.^{2} Epidermis is burned completely away. Dermis is markedly basophilic and completely coagulated, and bubble-like spaces extend halfway to surface. Severity scores of 4 and 5 were assigned to epidermis and dermis, respectively.

0.4, 1, and 8 sec., respectively. Since the white color gave way to gray as the energy was increased, the minimum energy for grayness was also the maximum for white. The appearance of scale did not preclude the simultaneous presence of gray; in fact, scale occurred only on the surface of a gray lesion. Data were also obtained for the energies at which transient erythema and charring appeared.

Histological Observations.—In Tables 1*A* and 1*B* the major pathological changes for the 24-hour postburn biopsies of the 0.4-sec. burns are briefly summarized and correlated with the gross observations. At 3.0 cal./cm.^{2} an increase in the number of erythrocyte-packed capillaries was seen near the surface of the dermis. With 3.5 to 4.0 cal./cm.^{2} the epidermis was pale-staining and the cells were swollen. The superficial dermis showed an increase in vascularity and a scattered infiltration of polymorphonuclear leucocytes. Figure 4*B* is a photomicrograph of a typical lesion of this type. A slightly more severe burn (Fig. 5*A*), 4.8 cal./cm.,^{2} showed a complete loss of epidermis and a replacement of the upper third of the dermis by an eosinophilic, structureless mass, beneath which there was an increased vascularity and a massive polymorphonuclear leucocyte infiltration. At 5.3 cal./cm.^{2} the epi-

TABLE 2.—*Comparative Severity Scores for Nine Approximately Equal-Energy Burn Series*[*]

Series	Exposure Time, Sec.	Energy, Cal./Cm.^{2}	Comparative Severity Scores			
			Epidermis	Dermis	Panniculus Carnosus	Total
A	0.1	3.0-3.6	1	2	0	3
	0.2	3.2-3.3	0	1	0	1
	0.5	3.0	0	1	0	1
	8.0	3.5-3.8	0	0	0	0
B	0.1	4.1-4.4	1	3	1	5
	0.2	4.3-4.6	1	*	1	4
	0.5	4.8	1	2	1	4
	0.8	4.5	1	2	1	4
	2.0	4.0-4.3	0	1	0	1
C	0.1	5.5-5.6	2	4	1	7
	0.4	5.3	2	3	1	6
	4.0	5.2-5.4	1	2	1	4
D	0.2	8.5-8.8	2	4	2	8
	0.4	8.2	2	4	2	8
	4.0	8.6-9.0	1	2	1	4
E	0.4	9.1	2	4	2	8
	8.0	9.1-9.6	2	3	1	6
F	0.8	10.6-10.7	2	4	2	8
	2.0	10.8-11.3	2	4	2	8
G	0.8	16.3-16.5	3	5	3	11
	8.0	16.5-17.1	2	4	2	8
H	0.4	20.5-23.9	3	5	3	11
	0.8	25.0	3	5	3	11
	4.0	20.6-22.5	2	5	3	10
	8.0	22.1-22.8	2	5	3	10
I	0.8	41.0	4	6	3	13
	2.0	38.3-40.5	3	5	3	11
	4.0	36.2-36.9	3	5	3	11
	8.0	36.8-40.0	3	5	3	11

[*] All biopsies taken at 24 hours.

dermis was thin and adherent to the dermis except for occasional small areas of separation, in which a material staining like serum was present. Except for pyknotic nuclei the epidermis was without visible cellular structure. The epidermis remained essentially without further change until the energy reached 12.5 cal./cm.,² at which its appearance was one of many tiny, exploded, empty blisters (Fig. 6*A*). The blisters became more fragmented with higher energies, and the entire epidermis is absent at about 24 cal./cm.² and above (Fig. 6*B*).

At 5.3 cal./cm.² the vascularity and polymorphonuclear leucocyte infiltration seen in the dermis at 3.5 to 4.8 cal./cm.² was diminished. Patent vessels and polymorphonuclear cells were not seen in the dermis (except at the extreme base) at energies of 7.0 cal./cm.² or higher (Fig. 5*B*). The dermal collagen showed a moderate degree of coagulation (or homogenization) with slight loss of its reticular structure at 5.3 cal./cm.² With higher energy levels these changes became more pronounced and extended deeper into the dermis, until at 12.5 cal./cm.² the entire dermis was coagulated and no normal reticular structure remained. At this energy the upper dermis had a splotchy, basophilic appearance, and the portion of the dermis immediately above the panniculus adiposus appeared frothy. Above 20 cal./cm.² the dermis became markedly thinned, hair shafts protruded from the surface, and the foamy character predominated in the deep half of the remaining dermis.

Slight nuclear pyknosis became apparent in the panniculus carnosus at 5.3 cal./cm.²; however, 7 to 8 cal./cm.² was required to produce visible vascular damage. Nuclear pyknosis and vascular damage became progressively more pronounced as the energy was increased. The milder vascular changes were usually accompanied by polymorphonuclear leucocyte infiltration. Above 20.5 cal./cm.² the panniculus carnosus was basophilic and coagulated.

In general there was a rough proportionality between the degrees of superficial and of deep damage in all the burns seen.

Based on the spectrum of histological changes of the 0.4-sec. series of 24-hour biopsies, arbitrary numerical grading systems were devised for the epidermis, the dermis, and the panniculus carnosus. The epidermal changes were assigned five grades ranging from 0 to 4; the dermis, seven grades from 0 to 6, and the panniculus carnosus, four grades from 0 to 3. These grades are described in Tables 1*A* and 1*B*. Each of the three layers of a burn lesion to be evaluated was matched with the description from this comparison series and the numerical grade of the description most applicable selected. The grades presented in Table 2 represent the average of those assigned to two, three, or four lesions of nearly the same incident energy and delivery time by two investigators. The lesions were grouped in series of approximately equal energies but of varying delivery times. In eight of the nine equal-energy series, the severity score is lower for the burns delivered over the longest period. A comparison of the scores within each of the three tissue layers of the nine equal-energy series shows that in every case a burn of longer delivery time received a score equal to or lower than the next more rapidly delivered burn in the series. For 18 of the 27 layers, the score is less for the longest exposure than for the shortest. Variation among the total scores within each series is less for the higher-calorie burns.

COMMENT

The spectrum of tissue damage found in the present radiant-energy, graded-severity, rat-skin burns ranged from transient erythema to actual charring deep in the dermal layer. Intermediate between these extremes successive increases in energy caused a stepwise change in the severity of the burn lesion. Relatively less severe burns showed evidence of inflammatory reaction, and as a consequence of the accompanying edema the margins of these lesions were difficult to distinguish until some time had elapsed and organization was established. Severer burns with immediate tissue coagulation appeared more discrete from the onset. Transition from a wet type of necrosis to dry necrosis was gradual and first occurred at the center of the lesion. These changes corresponded well with the descriptions of injury reported for burns of pig, dog, and guinea-pig skin produced by nonradiant energy sources. Thus, Elman and Lischer described reactions in dog skin following exposure to hot water varying from inflammation with edema to wet necrosis and finally to dry necrosis.[6] Leach, Peters, and Rossiter found cellular disintegration with mild burns and tissue coagulation with change in structure of collagen fibers with severer burns when guinea-pig skin was subjected to burns from a hot iron.[3] Similarly, Moritz in a study of the effect of hot water and hot air (to 900 C.) on pig skin reported that low-intensity burns showed a noncoagulative necrosis in which the involved tissue could undergo autolysis and organization; high intensities, however, resulted in coagulative necrosis with dead tissue disposed of by sequestration.[7] These comparisons indicate that burn damage is not fundamentally related to the manner of application of heat energy. They are consistent with the thesis that the extent of injury is a function of the temperature to which various portions of the tissue are raised and the time during which the temperature is maintained. The studies of Leach, Peters, and Rossiter show that within certain limits increasing the tissue temperature produces a greater degree of tissue injury. There is evidence that if the temperature is maintained for a sufficient time, the critical temperature for tissue injury is around 44 to 45 C.[1b]

Pearse, Payne, and Hogg described the pathological changes occurring in pig skin after exposure to a magnesium flash for 0.34 sec.[2] This skin exhibited the usual range of changes from erythema to dry necrosis. These authors stated that a characteristic of the flash burn is the method of healing, in that "The burned tissue represents a coagulative 'fixed' type of necrosis with eschar formation and subsequent sequestration rather than the organization in the non-coagulative necrotic tissue of the moderate temperature burn." The present evidence indicates that both types of healing occur in radiant-energy burns. The extent to which either process is represented in a particular burn depends on the amount and degree of damage done to the tissue. A long exposure of relatively low intensity can duplicate the effects of a short exposure of high intensity. As to severe burns with central eschar formation and subsequent sequestration, the present burn lesions showed a margin, both laterally and at the base, where the damage graded off imperceptibly to normal tissue and where organization appeared during the process of repair. It seems

6. Elman, R., and Lischer, C.: The Local Skin Lesion in Experimental Burns and Its Relation to Systemic Manifestations, Surg., Gynec. & Obst. **78**:346, 1944.

7. Moritz, A. R.: Studies of Thermal Injury: III. The Pathology and Pathogenesis of Cutaneous Burns: An Experimental Study, Am. J. Path. **23**:915, 1947.

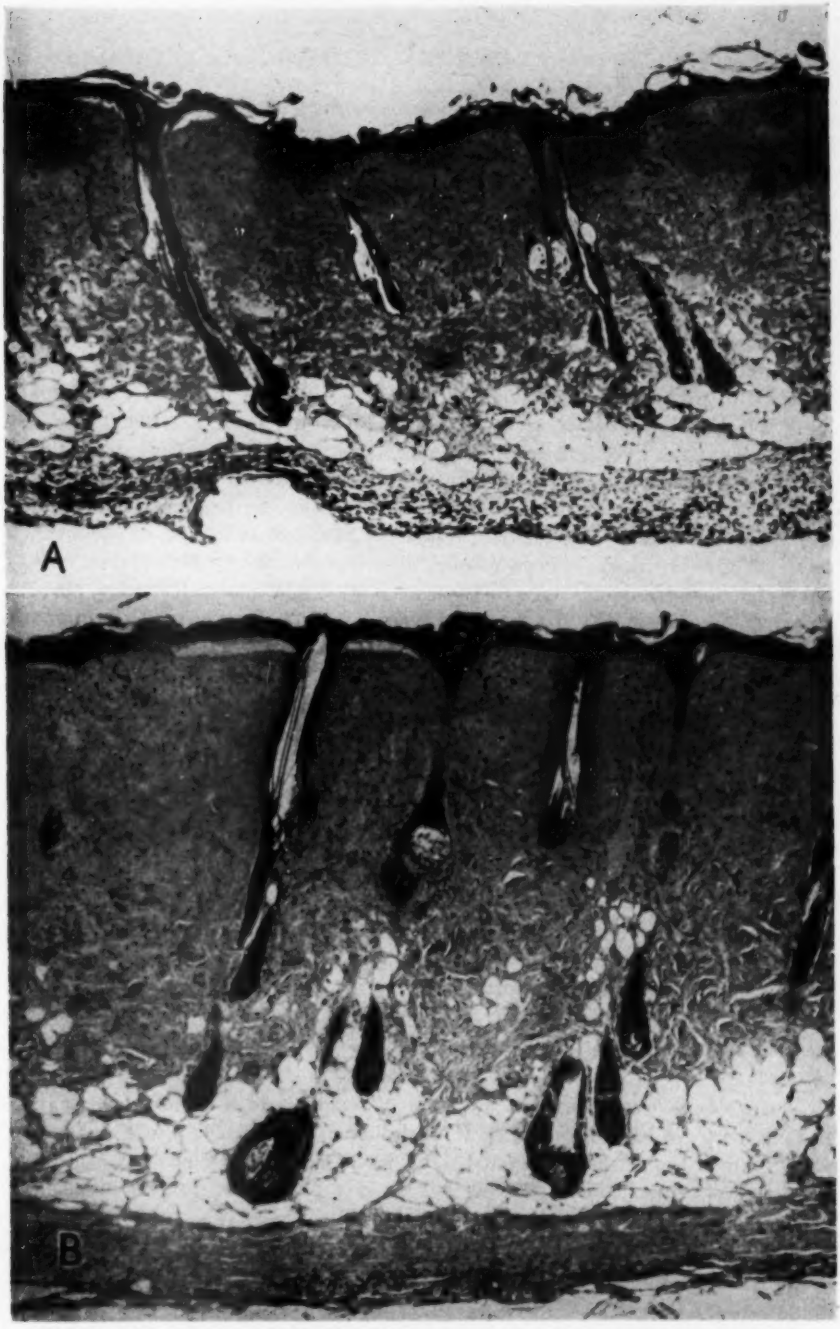


Fig. 7.—Effect of exposure time or delivery rate on resulting burn lesion. Hematoxylin and eosin stain; × 100. *A*, rat skin 24 hours after delivery of 7.0 cal./cm.^{2} during a 0.2 sec. exposure. Epidermis is thinned, basophilic, and without structure except for pyknotic nuclei. Dermis is completely coagulated and has several areas of increased basophilia. Epidermal-dermal separation is seen in one area. *B*, rat skin 24 hours after delivery of 11.2 cal./cm.^{2} during a 2.0 sec. exposure. Epidermis and dermis show slightly less basophilia than in *A*. There is more epidermal-dermal separation, and base of dermis shows less coagulation. These differences all favor interpretation that this lesion is somewhat less severe than that of *A*.

probable that differences between the injury produced by radiant energy and that produced by other types of thermal energy result from variations between the time-temperature curves for each level effected in the tissue rather than from the manner of energy application.

From the present studies on the gross and histological changes accompanying radiant-energy thermal burns, it is seen that the degree of tissue injury is related not only to the amount of energy delivered but is also a function of the rate of energy delivery.

Consideration of the gross pathology of the burn lesion as a function of energy delivered and delivery time points up the variation in the amount of incident energy required to produce a specific type of damage as the rate of delivery is changed (Fig. 3). Clear-cut differences were seen at the shorter times. The minimal energy which produced a gray lesion was 5.0 cal./cm.,^{2} 6.2 cal./cm.,^{2} and 8.4 cal./cm.^{2} for 0.1, 0.2, and 0.4 sec., respectively. Similar relations between threshold energy and exposure time were found for white and scaled lesions. At longer exposure times, 2 to 8 sec., the differences were less pronounced. The reason for the apparent trend, at longer exposure times, toward lessened dependence of the equally effective energy values on exposure time is not entirely clear. One would expect the energy requirement to increase sharply when the delivery time was long enough so that the incident energy could be dissipated by the tissue at a rate approaching the delivery rate.

Histological observation of several series of burns, each series produced by approximately equal amounts of energy delivered at differing rates, showed that less damage occurred when the exposure time was long, i. e., the delivery rate low. Similarly, a smaller amount of energy delivered in a shorter time could duplicate the action of a greater quantity delivered more slowly (Fig. 7). In an endeavor to assess more quantitatively the effect of varying delivery rate, the histological damage sustained by each skin layer was evaluated in terms of an arbitrary grading system. The results demonstrate that for a given energy the severity of these burns correlated with the energy intensity. The scores indicate a lessened dependence of tissue injury on delivery rate in the severer burns. This decreased dependence may be an artifact of the scoring system, since after a certain level of tissue damage was reached further variations in type of tissue change were limited and there was a tendency to grade all lesions alike.

SUMMARY

Burn lesions were produced by the application of high-intensity penetrating radiant energy, 3,100 A. to 22,000 A., to depilated rat skin. The gross and microscopic pathological changes so caused are described and correlated with changes reported for contact, flame, and hot-gas burns and with the amount of incident energy and rate of energy delivery.

The lesions were studied over a wide range of radiation intensities and exposure times. Some combinations of intensity and exposure time resulted in no detectable injury; others caused carbonization of the surface and complete coagulation of the skin and subcutaneous connective tissue. Many gradations between these extremes of severity were found, but there was no indication of other than a gradual transition from one degree of severity to another. The first perceptible injury to the epidermis was manifest by swelling and vacuolization of the cytoplasm and the disappearance

of nuclear structure. This amount of epidermal injury was accompanied by a mild inflammatory reaction in the dermis. At slightly higher energies, the epidermal-dermal cohesion was weakened and the epidermis slipped readily from the dermis. At such energies there was a massive inflammatory reaction of the dermis and a serous encrustation covered the surface of the lesion within a few hours. Still higher-energy burns showed thin, desiccated epidermal cells with markedly basophilic pyknotic nuclei; increasing amounts of dermal coagulation associated with a decreasing inflammatory reaction of the dermis, and a white-to-gray surface. The periphery of these lesions had the appearance of the milder burns. In the center of the severest burns the epidermis was burned away and the dermis was thin, completely coagulated, avascular, and basophilic. Coagulation and basophilia extended through the panniculus carnosus. Here again the periphery of the burn area showed gradations of lessened severity. The evidence of greater degrees of damage appeared first at the surface of the skin and then at progressively greater depths as the energy was raised.

From both gross and histological changes it was found that within the limits studied, 0.2 to 64 cal./cm.^{2}/sec. and 0.1 to 8.0 sec., increasing the rate of energy delivery reduced the amount of energy required to produce a specified degree of tissue injury. The evidence indicates that the tissue injury is a function of the temperature attained in the tissue and the duration of the temperature elevation rather than of the type of energy source.

EFFECTS OF HIGH INTENSITY RADIANT ENERGY ON SKIN

II. Quantitative Dependence of Tissue Injury on Duration of Exposure

E. L. ALPEN, Ph.D.
G. E. SHELINE, Ph.D., M.D.
P. R. KUHL, A.B.
AND
A. J. AHOKAS, A.B.
SAN FRANCISCO

IT HAS been pointed out that the degree of injury occurring in a burn lesion following contact with a hot object is related to the duration of contact and the temperature of the object.[1] Because of the nature of the heat-transfer process involved in this type of injury, efforts to quantitate the relationship between time, temperature, and injury have had indifferent success. With certain other biological effects of radiant energy, however, the result produced by the energy has been shown to be directly proportional to the product of intensity and radiation time. In the present study an investigation of the quantitative dependence of tissue injury on duration and intensity of radiant-energy exposure has been made.

Earlier studies in this laboratory indicated that for the production of equal-severity rat-skin burns with radiant energy (3,100A. to 22,000A.) a reciprocal relationship between time and intensity probably does not hold.[2] The evidence showed that the damage incurred by the skin depends not only on the total energy delivered but also on the duration of the radiation. These studies, although indicating a trend, left much to be desired. The differences observed were to a large extent based on subjective interpretation. To refine the definition of the time-intensity relationship, a dose-percent effectiveness method is applied in this study to analysis of the thermal burns produced by radiant energy.

From the Division of Biological and Medical Sciences, United States Naval Radiological Defense Laboratory.

This work was made possible, in part, by the Armed Forces Special Weapons Project and by the Bureau of Medicine and Surgery, Department of the Navy.

This article has been released for publication by the Division of Publications of the Bureau of Medicine and Surgery of the United States Navy. The opinions or assertions contained herein are the private ones of the authors and are not to be construed as official or reflecting the views of the Navy Department or the naval service at large.

1. Moritz, A. R., and Henriques, F. C., Jr.: Studies of Thermal Injury: II. The Relative Importance of Time and Surface Temperature in the Causation of Cutaneous Burns, Am. J. Path. **23**:695, 1947.

2. Sheline, G. E.; Alpen, E. L.; Kuhl, P. R., and Ahokas, A.: Effects of High Intensity Radiant Energy on Skin: I. Type of Injury and Its Relation to Energy Delivery Rate, A. M. A. Arch. Path., this issue, p. 265.

METHODS

Female Sprague-Dawley rats, depilated 45 to 60 days after birth, were burned on either the seventh or eighth day following depilation. The source of thermal radiation and the burning techniques used were described previously.[2] On each of several successive days 20 to 25 animals were burned. After burning, the lesions were photographed and the gross description recorded. At the appropriate time each lesion was scored for the presence or absence of the particular threshold of damage to be observed.

In the preceding paper descriptions were given of the lesions seen on rat skin after the application of various amounts of energy. The manner in which these lesions varied as the energy was increased was considered in detail. The two most sharply characterized of these lesions were, at lower energies, the presence of a white area (which, except for transient erythema and edema, is the first visible surface evidence of burn damage) and, at higher energies, the presence of multiple tiny empty vesicles or scales overlying an indurated gray area.

TABLE 1.—*Appearance of "Scale" Burn as a Function of Applied Energy (0.8-Second Exposure Time)*

Class Interval, Cal./Cm.^{2}	Center of Class Interval, Cal./Cm.^{2}	No. of Burns	Positive Response, %
8.0- 9.9	9.0	8	0
10.0-11.9	11.0	8	0
12.0-13.9	13.0	11	36
14.0-15.9	15.0	26	54
16.0-17.9	17.0	24	83
18.0-19.9	19.0	6	100
20.0-21.9	21.0	5	100
		N = 88	

Two dose-percent effectiveness studies were carried out. In the first study the presence or absence of a white area five minutes after exposure was investigated. The second study was a similar investigation with the presence or absence of "scale" five minutes after exposure to the radiant energy as the criterion of damage. At the time of observation the exact energy delivered was not known to the grader. The animals burned on a particular day were distributed among the various dose levels in such a fashion that each dose included data collected on several days. A chi-square test applied to the cumulative data indicated homogeneity of the results obtained on the several exposure days.

Calculation of the median effective dose (ED_{50}) and the confidence limits for this value were made according to the Litchfield and Wilcoxon [3] method for the Probit solution of the dose-percent response relationship. Because of the nature of the exposure technique, the data were grouped into class intervals of applied energy. The exact energy delivered to an animal was calculated after the exposure on the basis of data from a monitoring calorimeter. The amount of energy delivered varied somewhat from the predicted value because of small fluctuations in light intensity during exposure. For convenience of analysis, five to eight class intervals of uniform

3. Litchfield, J. T., Jr., and Wilcoxon, F.: A Simplified Method of Evaluating Dose-Effect Experiments, J. Pharmacol. & Exper. Therap. **96**:99, 1949.

size covering the range from the 0% response up to the 100% response were selected. Fifty to one hundred burns were included in the determination of each ED_{50}. All data were plotted on log probability paper and the ED_{50} determined graphically. The confidence limits stated are the 99% probability limit. Throughout this paper the median effective dose indicates the median applied energy, and no corrections have been made for reflection, scattering, or transmission. As an example, the results obtained for the 0.8 second exposure time of the "scale" burn series are shown in Table 1. The results for each exposure time of both the higher- and the lower-energy burns were similarly tabulated.

TABLE 2.—*Median Effective Dose for "White" Burn*

Exposure Time (t), Sec.	ED_{50}, Cal./Cm.²	Radiation Intensity, Cal./Cm.2/Sec.	$\frac{ED_{50}\ (t_0)}{ED_{50}\ (t_n)}$
0.08	3.3 ± 0.2*	41	1.5 ± 0.1*
0.1	3.4 ± 0.3	34	1.4 ± 0.2
0.3	4.0 ± 0.1	13.3	1.2 ± 0.1
0.8	4.2 ± 0.1	5.4	1.2 ± 0.1
8.0	4.9 ± 0.3	0.61	.....

* Confidence interval, 99%.

TABLE 3.—*Median Effective Dose for "Scale" Burn*

Exposure Time (t), Sec.	ED_{50}, Cal./Cm.²	Radiation Intensity, Cal./Cm.2/Sec.	$\frac{ED_{50}\ (t_0)}{ED_{50}\ (t_n)}$
0.13	9.8 ± 0.2*	75.5	3.6 ± 0.1*
0.4	12.0 ± 0.5	30.0	3.0 ± 0.1
0.8	14.5 ± 0.4	18.1	2.4 ± 0.1
2.0	21.0 ± 0.6	10.5	1.7 ± 0.1
4.0	29.0 ± 0.5	7.2	1.2 ± 0.1
6.0	35.5 ± 1.0	5.9	.....

* Confidence interval, 99%.

RESULTS

The calculated values for the median effective dose, ED_{50}, for each exposure time investigated for both "white" and "scale" burns are given in Tables 2 and 3, respectively. For "white" burns the ED_{50} increased progressively from 3.3 ± 0.2 cal./cm.² to 4.9 ± 0.3 cal./cm.² as the exposure was increased from 0.08 to 8.0 sec. A similar but more pronounced trend in the ED_{50} values is seen for the "scale" burns, for which, over an exposure range of 0.13 to 6.0 sec., the ED_{50} rose from 9.8 ± 0.2 to 35.5 ± 1.0 cal./cm.² In covering these ranges of exposure, radiation intensities varying from 0.61 to 75.5 cal./cm.²/sec. were studied (Tables 2 and 3). Charts 1 and 2 demonstrate the more abrupt rise in ED_{50} values for "scale" as compared with "white" burns. The vertical lines in these charts represent the 99% confidence interval for each value graphed. It is readily apparent that the differences between the ED_{50} values are significant.

For both burn series, the ratio between the ED_{50} for the maximum exposure and the ED_{50} for each other exposure was calculated (Tables 2 and 3). The values for this ratio indicate the effectiveness of a given radiation intensity as compared

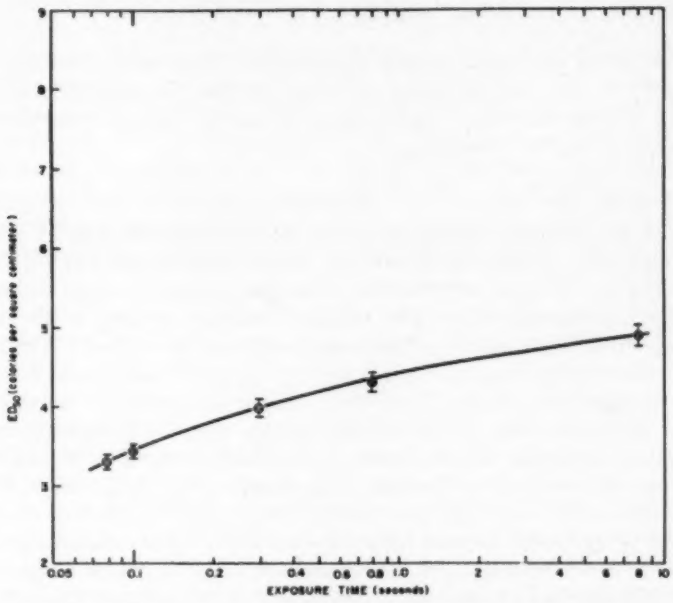


Chart 1.—Median effective dose (ED_{50}) for a "white" burn. Variation with exposure time, and indirectly with radiation intensity, of incident energy required to produce a given tissue effect is demonstrated.

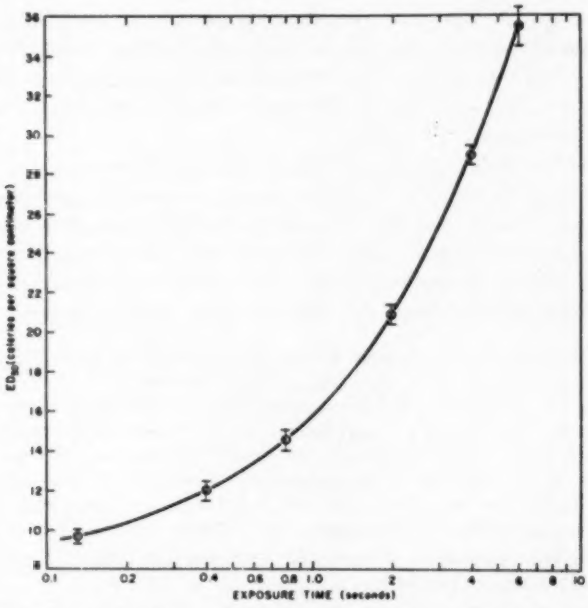


Chart 2.—Median effective dose (ED_{50}) for a "scale" burn. Variation with exposure time, and indirectly with radiation intensity, of incident energy required to produce a given tissue effect is demonstrated.

with that for the minimum intensity studied. The effectiveness ratio for "white" burns is 1.5 ± 0.1 when the ED_{50} at 41 cal./cm.²/sec. is compared with that at 0.61 cal./cm.²/sec. For "scale" burns an intensity of 75.5 cal./cm.²/sec. is 3.6 ± 0.1 times as effective as an intensity of 5.9 cal./cm.²/sec.

COMMENT

Several authors have reported studies on the relationship of duration and intensity of ultraviolet radiation to germicidal action, erythema production, or other measures of the biological effectiveness of radiant energy.[4] These workers have found that a reciprocal relationship between time and intensity holds for these biological effects in the time intervals studied, microseconds to hours. The relationship, as formulated by Luckiesh,[5] is that for a given survival ratio of micro-organisms exposed to germicidal energy, the product of intensity multiplied by duration of exposure is constant. The product of intensity and time is total energy delivered; therefore the relationship can be restated as, the biologic effect is determined by the total energy delivered. This relationship has been referred to by Waller [4a] as the Bunsen-Roscoe law. The law, as originally stated, applied to the photochemical absorption of light only. In these studies it is conceivable that with the wave length of radiation used, a photochemical mechanism was involved. In such situations the quantal yield could be expected to be independent of the radiation intensity and of the temperature attained in the tissues. If a reciprocal relationship existed between exposure time and intensity (i. e., effect is proportional to intensity times exposure time) for the thermal burns studied, the effectiveness ratio ED_{50} $(t_{max})/ED_{50}$ (t_n) would be independent of radiation intensity and would have a value of 1.0 for all exposure times. In every case, in both series, the ratio of the ED_{50} for exposures of less than the maximum to the ED_{50} at the maximum time studied is greater than 1.0 (Tables 2 and 3). In fact, this ratio increases progressively as the exposure time is shortened, indicating greater effectiveness for burn production as the energy is delivered during a shorter period.

Henriques [6] derived a rate expression for thermal death of tissue. It was concluded that thermally induced epidermal injury is primarily due to denaturation of certain tissue proteins and that the amount of damage incurred is a function of the temperature attained in the tissue and the duration of the hyperthermic episode. Since in the present experiments neither the surface-temperature nor the trans-epidermal-temperature gradient was determined, it was not possible to apply a

4. (*a*) Waller, M. D.: The Relation Between Energy Doses of Ultraviolet Radiation and Actinic Erythema Produced, Proc. Roy. Soc., London, s. B **108**:264, 1931. (*b*) Luckiesh, M., and Taylor, A. H.: Erythemal and Tanning Effectiveness of Ultraviolet Energy, Gen. Elect. Rev. **42**:274, 1939. (*c*) Blum, H. F., and Gilbert, H. W.: Studies of Photodynamic Hemolysis with Monochromatic Light: The Reciprocity Law, J. Cell. & Comp. Physiol. **15**:75, 1940. (*d*) Rentschler, H. C.; Nagy, R., and Mouromseff, G.: Bactericidal Effect of Ultraviolet Radiation, J. Bact. **41**:745, 1941. (*e*) Rentschler, H. C., and Giese, A. C.: Injury and Death Resulting from Microsecond Flashes of Ultraviolet Light, J. Cell. & Comp. Physiol. **17**:395, 1941.

5. Luckiesh, M.: Applications of Germicidal, Erythemal and Infrared Energy, New York, D. Van Nostrand Company, Inc., 1946.

6. Henriques, F. C., Jr.: Studies of Thermal Injury: V. The Predictability and the Significance of Thermally Induced Rate Processes Leading to Irreversible Epidermal Injury, Arch. Path. **43**:489, 1947.

calculation similar to that of Henriques to these radiant-energy burns. However, if tissue damage from this type of radiation is a function of the duration of the hyperthermic episode and the temperature gradients established, then one would not expect, except possibly for very short times, to approach a reciprocal relationship between radiation intensity and time of delivery. This assumption follows, since time favors the dissipation of heat away from the burn area. That the present data do exhibit a dependence upon delivery rate is in accordance with the explanation that this type of energy gains its effect through elevation of the tissue temperature.

The observations reported here pertain only to the surface of the burn lesion. In the preceding paper evidence was given that a correlation exists between the amount and depth of tissue injury and the extent of surface damage.[2]

SUMMARY

This report deals with the relationship between exposure time and radiation intensity of radiant thermal energy in producing a given surface lesion. It is demonstrated for burns produced by concentrated radiation from a carbon arc source that the relation between radiation intensity and duration required to produce a given degree of injury to the skin is not a simple reciprocal function. The deviation from a reciprocal relationship is evaluated quantitatively. Over the dose and intensity ranges studied the shorter the exposure time or the greater the radiation intensity, the smaller the dose required to produce the effect. The results are in agreement with the concept that thermal injury is primarily due to protein denaturation.

MILK-ASPIRATION PNEUMONIA IN HUMAN AND ANIMAL SUBJECTS

THOMAS J. MORAN, M.D.
PITTSBURGH

PNEUMONIA caused by aspiration of milk, usually classified as a form of lipid pneumonia, is a recognized complication in pediatric practice, but it has not been considered a disease of importance in adult life. Autopsy studies of both infant and adult subjects have convinced me that this disease is more common than most clinicians and pathologists realize. Lack of recognition of the disease by pathologists is due partly to the facts that identification of milk in tissue sections is difficult and that the pathologic changes produced by aspiration of milk have not been completely understood.

In autopsies at the St. Margaret Memorial Hospital, milk-aspiration pneumonia has been found frequently in infants who had feeding problems due to prematurity, central nervous system defects, or obstruction of the esophagus. Aspiration of milk accounted for sudden death in one apparently healthy infant, 3 months old, who had been allowed to take his feeding mixture with his bottle propped against a pillow without help or observation of the mother. In adults this disease has been found most frequently in persons confined to bed by other diseases or injuries, in old people with impaired reflexes, and in mental patients. It may be found in association with the lipid pneumonias caused by liquid petrolatum or with the aspiration pneumonias produced by solid foods. The disease may result from direct aspiration of milk or from aspiration of vomitus containing milk.

The clinical diagnosis of milk-aspiration pneumonia is difficult. A history of difficulty in swallowing or of choking during feedings may often be obtained by questioning persons responsible for feeding the patient. This observation is true especially in regard to premature infants. A history of gavage feeding is common for infants with this type of pneumonia. Both infant and adult patients with this disease show resistance to antibiotic therapy. Otherwise the clinical features of milk-aspiration pneumonia are essentially the same as those of pneumonia caused by other agents.

INCIDENCE

A statistical survey of the autopsy incidence of milk-aspiration pneumonia has not been attempted, but five instances of this disease have been found among the last 43 autopsies at the St. Margaret Memorial Hospital. Three of these, which occurred in infants, accounted for the death of the patient. The oldest of the three babies died 14 days after birth. A congenital goiter weighing 12 gm. had compressed

From the Department of Pathology and the John C. Oliver Memorial Research Foundation, St. Margaret Memorial Hospital, and the Department of Pathology, University of Pittsburgh School of Medicine.

the esophagus, resulting in aspiration of milk into the trachea. This case has previously been reported in detail.[1] One premature baby requiring gavage feeding died 10 days after birth. One baby with a tracheoesophageal fistula died five days after birth and one day after repair of the fistula. The esophageal and tracheal repair sites were intact, but an extensive milk-aspiration pneumonia resulted in the baby's death. Milk had been given this baby before the diagnosis of a tracheoesophageal fistula had been established.

In the two adult subjects, the milk-aspiration pneumonia was contributory to the death but was not the principal cause of death. One patient was a 60-year-old man with rheumatic heart disease and congestive heart failure. He had been on a liquid diet, principally milk, for several weeks prior to his death. The other patient was an 81-year-old man with cerebral softening, arteriosclerotic heart disease with coronary thrombosis, and a fracture of the left femur. The cerebral softening had been present before his admission to the hospital and had caused difficulty in speech and swallowing. Milk had been one of his principal foods for several weeks, and he had choked on it on several occasions.

GROSS FEATURES

The gross diagnosis of milk-aspiration pneumonia cannot be made unless the process is acute and the milk is found in the bronchi. Milk in the bronchi can easily be overlooked when the bronchi and pulmonary vessels are cut, as the milk is quickly obscured by blood entering the bronchi. The trachea should be tied before the lungs are removed if aspiration of milk seems likely. When milky fluid is found, direct smears of the material stained with Sudan IV should be examined for fat globules.

Grossly, the affected lungs show bronchopneumonia, which is often hemorrhagic and often associated with areas of emphysema. The dependent portions of the lungs are usually most widely involved, as is true in other forms of aspiration pneumonia. The involved portions of the lung may be firm and solid, or they may be rubbery and irregularly hemorrhagic. The firm or rubbery hemorrhagic areas may resemble areas of collapse. In a few instances the affected portions may become liver-like in consistency. Several examples of unilateral or bilateral lobar pneumonia have been encountered both in infants and in adults. Occasional abscesses were found in lungs showing a lobar type pneumonia.

MICROSCOPIC FEATURES

The microscopic features of milk-aspiration pneumonia are varied, but they are characteristic enough to permit at least a tentative diagnosis in a high percentage of cases. The pneumonic reaction is essentially mononuclear, and it may be entirely mononuclear unless death occurs early in the course of the disease. In early stages of the disease the milk may be seen in the bronchi and in the alveoli as amorphous pink-staining material resembling fibrin. The material may be plastered against the alveolar walls and the bronchi in such fashion as to resemble the hyaline membranes seen in autopsies on newborn infants or on older infants and adults who have died

1. Moran, T. J.: Congenital Goiter with Death from Milk-Aspiration Pneumonia: Report of a Case with Autopsy, A. M. A. Arch. Path. **54**:213, 1952.

of virus pneumonia. The milk may easily be overlooked unless the pathologist is familiar with its appearance and with the surrounding reaction in the tissues.

The earliest response to the aspirated milk is exudation of red blood cells, segmented neutrophiles, and macrophages into the bronchi and alveoli. An acute pneumonic reaction occurs within a few hours after aspiration of the material. Varying numbers of large, pale, foamy, finely vacuolated macrophages resembling the lipophages of lipid pneumonia caused by liquid petrolatum are seen in the pneumonic areas even within the first few hours. The vacuoles in these cells are not usually so large and so sharply defined as those seen in liquid-petrolatum-

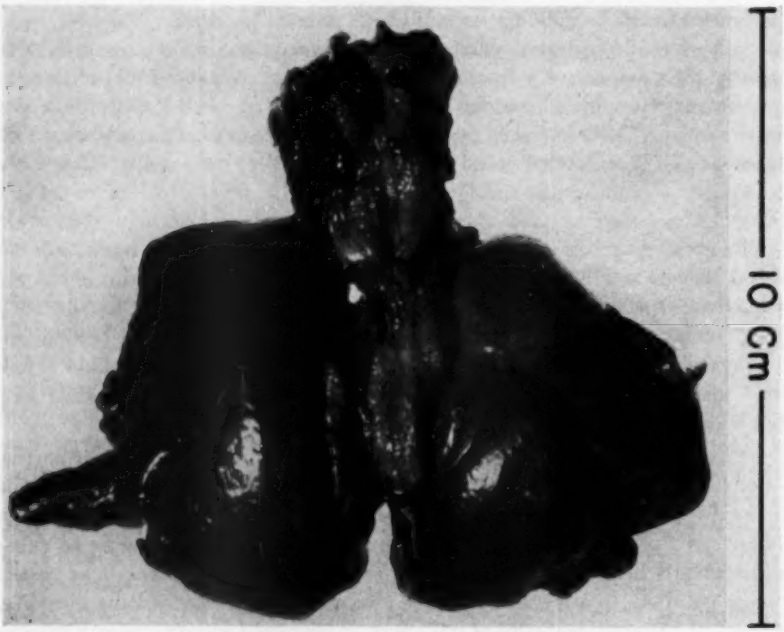


Fig. 1.—Extensive pneumonia following aspiration of milk in 5-day-old infant with tracheo-esophageal fistula. Fistula repair site is intact.

aspiration pneumonia. These cells are often seen around pink-staining masses of milk in the alveoli. Even in early stages they contain deeply sudanophilic globules.

Within a few days the reaction becomes granulomatous, with exudation of numerous mononuclear cells into the alveolar spaces, thickening of the alveolar walls, and formation of small focal collections of "epithelioid" cells. Giant cells, occasionally large and bizarre, may be seen in varying numbers in this stage. These giant cells may have vacuolated spaces, apparently caused by fat, or they may contain bits of the milk protein. The giant cell reaction is not constant, and its absence cannot be used as a means of eliminating the possibility of this disease. The granulomatous foci become extensive, and small focal collections of "epithelioid" cells and giant cells may give an appearance superficially similar to that of Boeck's sarcoid or tuberculosis. Bits of milk in the center of these collections of cells may

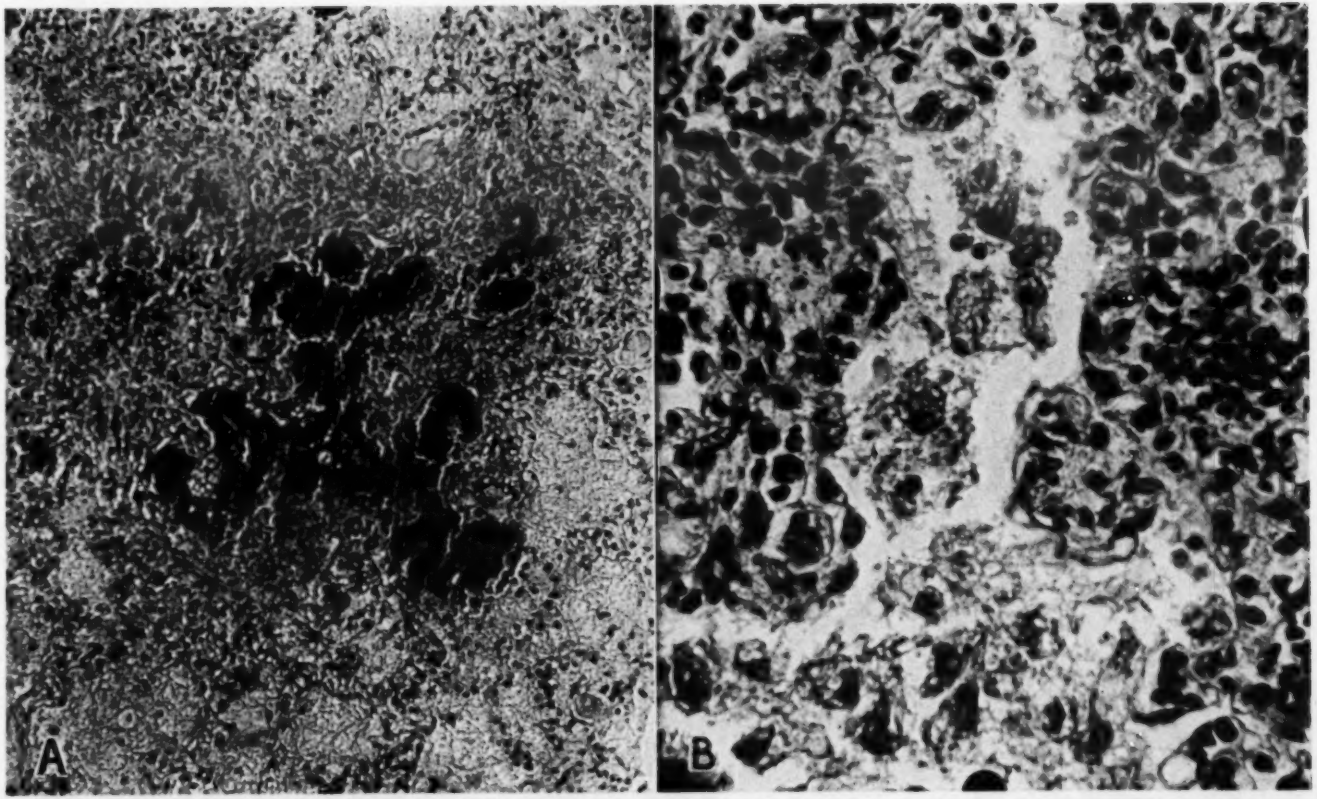


Fig. 2.—*A*, early hemorrhagic pneumonia in 1-month-old infant who died two days after aspiration of feeding mixture. Feeding mixture appears as dark-staining material in center; × 150. *B*, mononuclear pneumonia in 14-day-old infant. Large macrophages are seen in central space; × 1,000.

simulate necrotic foci. Varying numbers of segmented neutrophiles may be mixed with the macrophages in the alveolar spaces. Areas of emphysema alternate with the granulomatous foci or with focal areas of collapse.

As the lesions progress, the granulomas become larger, alveolar thickening is more prominent, giant-cell reaction may or may not be prominent, emphysematous areas are larger and more numerous, and early fibrosis occurs. Fibrinous and fibrous pleurisy may be seen over granulomatous foci in subpleural spaces. In this stage the milk is rarely identifiable in the sections.

In those cases in which repeated aspirations of milk have occurred, all stages of the process may be seen in the lungs at the same time. In such instances the giant-cell reaction may be a striking feature of the disease.

Frozen sections stained with Sudan IV show the presence of sudanophilic globules, usually small, in the macrophages and occasionally in giant cells. These fat-containing cells are more prominent in early stages of the disease. In early stages the fat globules are also seen free in the tissues, and in late stages they may be seen in granulomatous or fibrotic areas.

Tissue reactions to feeding mixtures, human milk, and cow's milk could not be differentiated in our autopsy experience.

EXPERIMENTAL MILK-ASPIRATION PNEUMONIA

Because of the difficulty encountered in the definite identification of milk in histologic preparations, and because of the variations in tissue response in different human subjects, it seemed advisable to undertake an experimental study of the effects of aspiration of milk on the lungs of rabbits and guinea pigs. The gross and microscopic findings in the first few rabbits and guinea pigs were identical. Because of the greater ease of intratracheal injection in rabbits, the use of guinea pigs was discontinued, and this report is limited to a description of the changes produced in rabbits.

Methods.—Varying amounts of fresh human milk, cow's milk, or feeding mixture (8 oz. evaporated milk, 20 oz. distilled water, and 4 tablespoons of dextrimaltose[3] No. 1) were injected intratracheally into 50 rabbits. Only one type of milk was injected into each animal. The injections were given with aseptic technique with the animal under ether anesthesia. After injection of the milk the animal was held in position on its feet so that most of the material drained into normally dependent portions of the lungs. Each of two animals was given 3 cc. of feeding mixture from which the fat had been removed by alcohol and ether extractions. Some animals received single injections of 2.5 cc. to 3 cc. of milk, and some were given 3-cc. or 4-cc. injections on three successive days. Other animals were given single injections of large amounts of milk (5 to 70 cc.). The animals died or were killed by chloroform or venous air injections at intervals varying from two minutes to two months after aspiration of the milk (Table). The amount injected into many of the animals was small, so that most of them did not die of the pneumonia produced and were available for study of the changes in the lungs at various times after injection. Numerous lungs of normal rabbits killed by chloroform or air injection were available for control studies.

Results.—No appreciable differences were found in the tissue responses to feeding mixture, human milk, and cow's milk. Some of the changes were similar to those produced by aspiration of liquid petrolatum or cod liver oil and were undoubtedly produced by the milk fat. The general response, however, closely resembled the changes observed in human food-aspiration pneumonia[2] and experimental food-

aspiration pneumonia in guinea pigs,[2] and the disease produced could not be regarded as a form of lipid pneumonia. The removal of the fat from the milk by repeated alcohol and ether extraction did not affect the reaction produced in two animals given injections of this material except that fat globules were not demonstrated in the macrophages.

The microscopic changes included exudation of red blood cells, granulocytes, and macrophages; acute pneumonia with abscess formation; mononuclear pneumonia; chronic granulomatous reaction, and fibrosis. Giant-cell reaction was striking in a few instances, slight to moderate in many, and entirely absent in others. Emphysema and collapse were prominent features in many of the affected portions of the lungs.

Animals Grouped According to Method of Injection of Milk and Time of Death

	Total Animals	Died	Killed
Single injections of 3 cc.; all types of milk	11	0	2 min. (1), 5 min. (1) 30 min. (2), 2 hr. (2) 6 hr. (2), 2 days (1) 3 days (2)
Injections of 3 cc. or 4 cc. on 3 consecutive days; all types of milk	15	27 days (1) 39 days (1)	9 days (1), 11 days (1) 14 days (1), 17 days (1) 1 mo. (2), 6 wk. (2) 2 mo. (5)
Repeated 5-cc. injections (17 times in 22 days); feeding mixture	1	23 days (1)	0
Injections of large amounts (10 cc. or more *); feeding mixture	11	20 cc. (1 min.), 10 cc. (2 min.) 20 cc. (2 min.), 20 cc. (5 min.) 15 cc. (10 min.), 30 cc. (11 min.) 10 cc. (12 hr.), 35 cc. (24 hr.)	15 cc. (3 days) 10 cc. (4 days) 10 cc. (14 days)
Injections of large amounts (10 cc. or more *); cow's milk	10	20 cc. (4 min.), 30 cc. (35 min.) 40 cc. (35 min.)	60 cc. (1 hr.), 55 cc. (1 hr.) 50 cc. (1 hr.), 50 cc. (1 hr.) 12 cc. (1 hr.), 15 cc. (1 hr.) 70 cc. (3 hr.)
Injections of large amounts (10 cc. or more *); human milk	2	0	20 cc. (30 min.) 15 cc. (1 hr.)

* Amounts larger than 20 cc. could not be injected at one time and were given in divided doses at 15-minute or 30-minute intervals.

An attempt was made to determine whether any reaction to the milk occurred within the first few minutes after injection. Several animals given large and small injections of milk (2.5 to 30 cc.) were killed in 2 to 10 minutes. Other animals were given various amounts (5 to 70 cc.) of human milk, cow's milk, or feeding mixture to determine whether or not injections of large quantities of milk would produce death. Relatively small quantities (10 to 20 cc.) of feeding mixture often produced death from pulmonary edema in 1 to 11 minutes, while relatively large amounts (20 to 70 cc.) of cow's milk or human milk killed only 3 of 10 animals in 4 to 35 minutes. This difference in reaction was due to the increased amounts of carbohydrate present in the feeding mixtures. Results of these experiments will

2. Fetterman, G. H., and Moran, T. J.: Food Aspiration Pneumonia, Pennsylvania M. J. **45**:810, 1942.

3. Moran, T. J.: Experimental Food-Aspiration Pneumonia, A. M. A. Arch. Path. **52**:350, 1951.

be reported separately,[4] but the lungs of the animals that died within a few minutes and those that survived these amounts were available for this study.

The gross and microscopic observations on the animals that died or were killed 2 to 10 minutes after aspiration of the milk were of interest. When the bronchi were opened, fluid and curdled milk was found. The bronchi were injected and dusky. The lungs showed no definite changes except increased weight in some instances and occasional petechial hemorrhages. Smears were made of the milk in the bronchi without disturbing the mucosal surface, and bits of the curdled milk were picked out with forceps. Some of the curdled material was smeared, and some was fixed

Fig. 3.—Rabbit lungs four days after injection of 10 cc. of milk. Pneumonia with partial collapse in one lung, pneumonia with emphysema in other.

in 4% formaldehyde and put through as routine paraffin sections stained with hematoxylin and eosin. The smears were stained with hematoxylin and eosin and Sudan IV.

Both the smears and the sections of the milk showed numerous red blood cells, granulocytes, and macrophages mixed with the milk. In the tissue sections even from the animals that died within two minutes, occasional macrophages contained vacuoles. In smears of the material stained with Sudan IV there were occasional sudanophilic globules in the macrophages.

4. Moran, T. J.: Experimental Production of Acute Pulmonary Edema by Intratracheal Injection of Milk, Feeding Mixture, and Carbohydrates Used in Feeding Mixtures, to be published.

In the case of animals killed 2 to 10 minutes after aspiration of the milk, routine tissue sections of the lungs stained with hematoxylin and eosin showed large quantities of pink-staining or occasionally pale-blue-staining milk in the alveolar spaces and in the bronchi. In several areas the application of the milk against the bronchial and alveolar walls gave an appearance resembling the hyaline membranes of virus pneumonia. Numerous granulocytes were present in the alveolar capillaries, and numerous macrophages were applied to the alveolar walls. In many areas numerous red blood cells and occasional collections of granulocytes and macrophages were mixed with the milk in the alveoli. Occasional bronchi contained red blood cells,

Fig. 4.—Pneumonia and emphysema five days after injection of 3 cc. of milk into rabbit.

granulocytes, and macrophages. Many alveoli were ruptured. Frozen sections of the lungs stained with Sudan IV showed large quantities of faintly sudanophilic material in the bronchi and alveoli. Numerous orange-to-red fat globules were seen in the aspirated material. A few macrophages contained sudanophilic globules.

Because of the unexpected rapidity of this tissue response, one animal was given an intravenous injection of 20 cc. of air until respirations ceased, and 20 cc. of cow's milk was immediately injected into the trachea. Although from external examination the animal appeared dead, the heart was still beating irregularly when the lungs were removed. Smears and sections of the milk from the bronchus showed frequent red blood cells and a few granulocytes and macrophages. Tissue sections

of the lungs also showed red blood cells and a few granulocytes and macrophages in the alveoli and bronchi. The earliest inflammatory response must occur, then, in a matter of seconds rather than of hours.

Study of the lungs of control animals killed by air embolism and by chloroform inhalation showed frequent granulocytes in the alveolar capillaries and occasional macrophages applied to the alveolar walls but not in numbers comparable to those in the lungs containing milk. A few macrophages were seen in alveolar spaces, but red blood cells and granulocytes were rare. Red blood cells and granulocytes were not seen free in the bronchi in appreciable numbers.

Animals killed 30 minutes after injection of milk showed gross changes in the lungs, with irregular, firm, hemorrhagic areas, especially in the dependent portions. These animals showed an early pneumonic reaction, with red blood cells, granulocytes, and macrophages in the alveolar spaces and in the bronchi, especially around the milk.

Lungs of animals killed at two hours also showed irregular, firm areas, especially in dependent portions. Numerous red blood cells, granulocytes, and macrophages were seen. Many of the macrophages at this stage were large, pale, and foamy.

In six hours a definite, extensive, hemorrhagic pneumonia was seen, with red blood cells, granulocytes, and macrophages around the milk in the alveolar spaces and in the bronchi. The pneumonic reaction was also seen in many alveoli which did not contain milk. Frozen sections stained with Sudan IV showed that many of the macrophages contained orange or red fat globules.

Two days after a single injection of 3 cc. of milk, an extensive acute pneumonic reaction was found, with numerous red blood cells, granulocytes, and macrophages in the alveoli and bronchi. At this stage a widespread granulomatous reaction was also seen, with numerous macrophages and occasional giant cells. The individual alveolar walls appeared thickened because of the adherence of numerous large, pale mononuclear cells resembling "epithelioid" cells. These cells often appeared to be connected even when only a single layer of cells was applied to the alveolar wall. In a few areas these cells were seen in double or triple rows, with irregular extension into the alveolar spaces. Recognition of definite milk particles was sometimes difficult at this stage.

After three days animals given injections of 3 cc. of milk showed hemorrhagic, firm, nodular areas alternating with emphysematous areas and normal-appearing lung tissue. In one animal given an injection of 15 cc. of feeding mixture, the dependent portions of the lungs were entirely solid, and the upper portions were irregularly hemorrhagic and rubbery. Microscopically, occasional areas of acute pneumonia with numerous granulocytes were seen, but most of the reaction was mononuclear. Numerous macrophages were seen in the alveolar spaces and in the interstitial tissue. Many alveolar walls were thickened. Under low-power magnification the alveolar thickening could easily be overlooked. Many alveolar walls were thickened by multiple, irregular layers of mononuclear cells, which appeared to be connected rather than deposited separately. Many of the collections coalesced, forming small granulomatous foci involving several alveoli. Some of these foci had merged to form large granulomas, which occupied as much as one-half of the lung tissue in the sections. Numerous granulocytes and red blood cells were sprinkled through these granulomas. Occasional multinucleated giant cells were

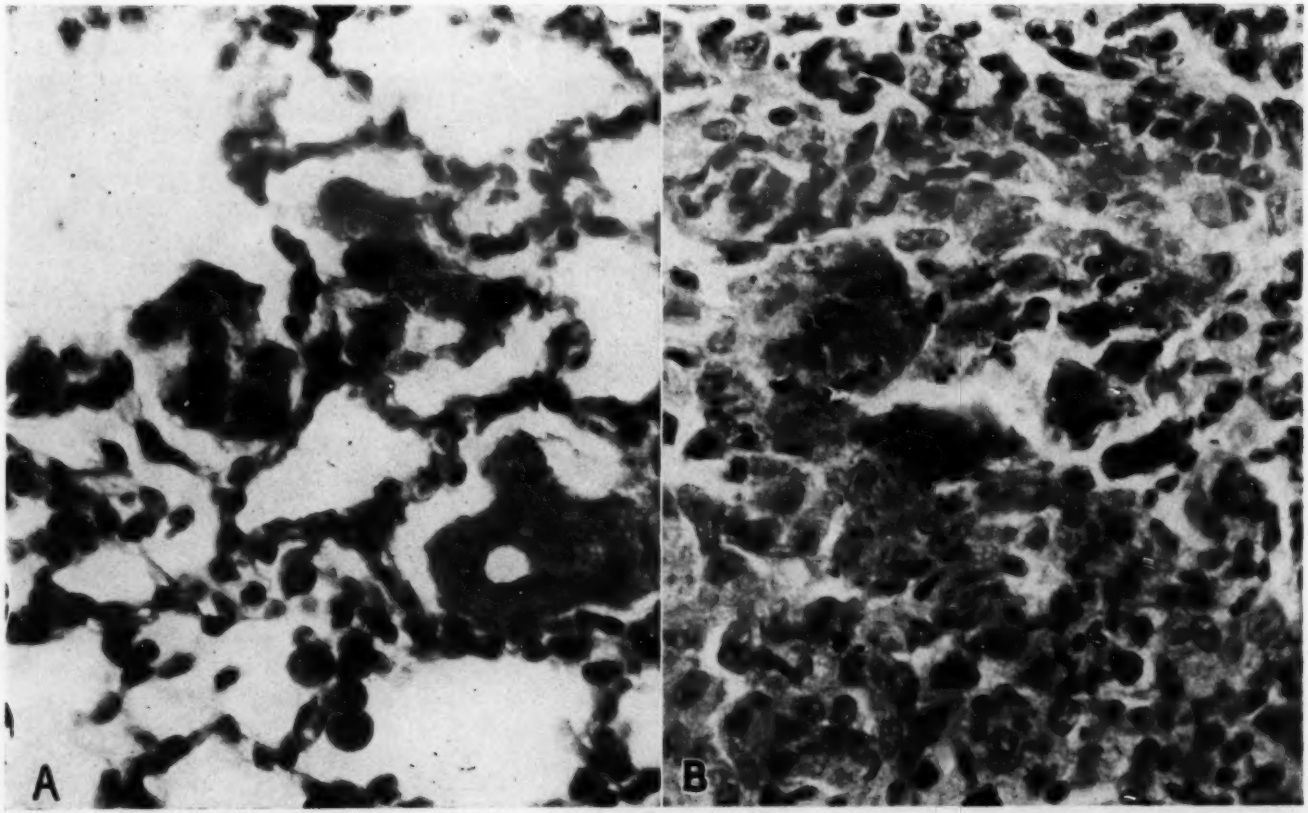


Fig. 5.—*A*, rabbit lung five minutes after injection of 3 cc. of milk. Milk is seen in alveolar spaces along with macrophages and granulocytes; × 900. *B*, mononuclear pneumonia in rabbit three days after injection of 3 cc. of milk. Milk is seen as amorphous material in center; × 900.

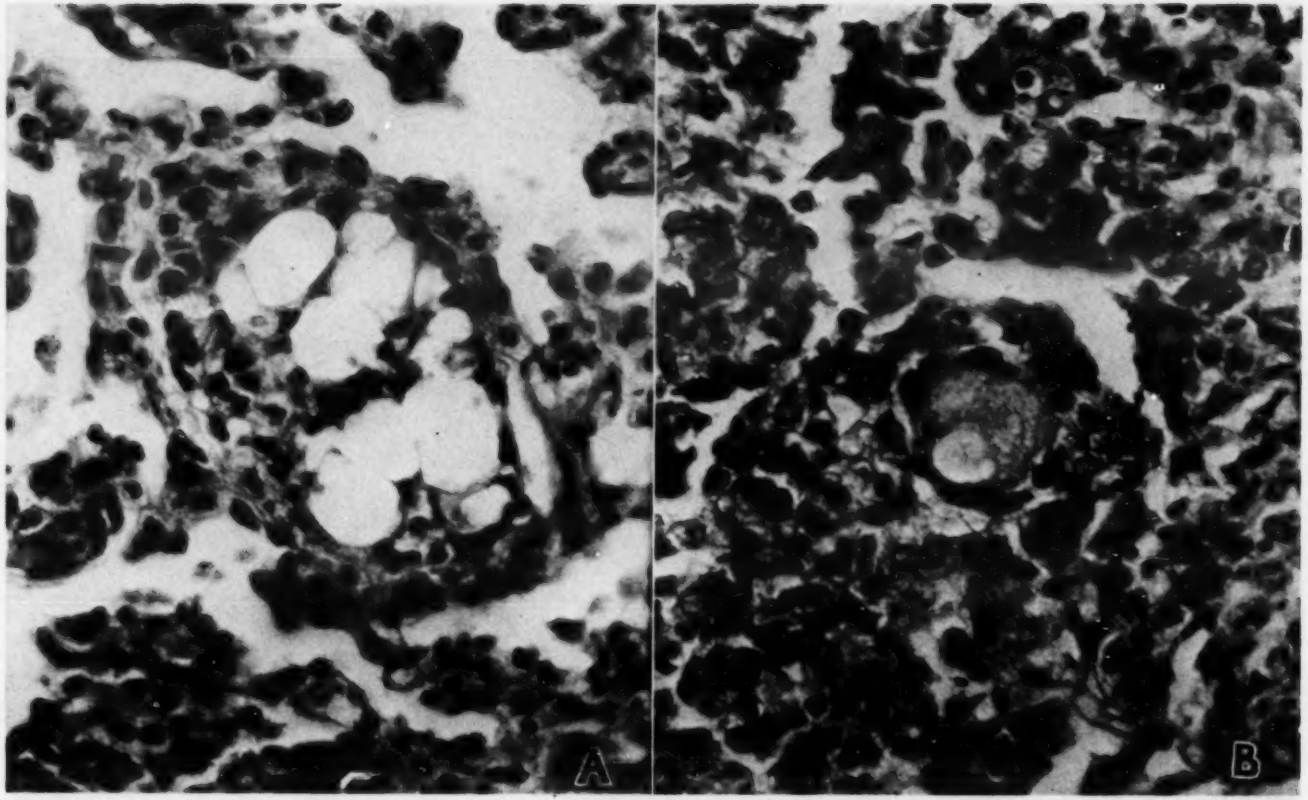


Fig. 6.—*A*, granuloma around coalesced fat globules in rabbit lung three days after injection of 5 cc. of milk; × 900. *B*, rabbit lung 23 days after injection of milk. Giant cell containing fat globule and amorphous material resembling milk protein. Giant cell is surrounded by early granulomatous reaction; × 900.

seen. Certain granulomas were formed around fat globules, some of which appeared to have coalesced in a manner similar to that seen in late liquid-petrolatum-aspiration pneumonia. Other granulomas were formed around pink-staining masses of milk, which did not contain fat. Occasional hemorrhages were found. Emphysema was present, not only near the large granulomatous areas and areas of focal collapse but also in areas showing only thickened alveolar walls and tiny granulomas.

Many of the animals kept longer than four days were given injections of 3 cc. or 4 cc. of milk daily for three consecutive days to see whether repeated aspirations altered the character or intensity of the reaction.

Animals given injections of 3 cc. or 4 cc. of homogenized milk on three successive days and killed at 9 to 11 days, grossly showed irregular rubbery, purplish-red areas especially in the dependent portions and occasional small emphysematous areas in all portions of the lungs. Most of the lung tissue on gross examination appeared normal. Microscopic examination, however, revealed an extensive granulomatous reaction varying from thickening of individual alveolar walls to large granulomas involving most of the lung tissue in the slide. These granulomas contained occasional granulocytes and occasional multinucleated giant cells. Many granulomas were located in subpleural spaces. The overlying pleura was often greatly thickened and contained numerous macrophages and occasional granulocytes. Early fibrosis was seen in the pleural reaction and in a few of the granulomas. Occasional small bits of material resembling milk were found in these sections.

Two animals were killed at 14 days. One had been given 3 cc. of feeding mixture on three consecutive days and the other 3 cc. of cow's milk on three consecutive days. The lungs of both showed irregular, rubbery, hemorrhagic areas. Microscopically an extensive granulomatous reaction with early fibrosis was seen. No milk could be identified. Animals killed 17 days after three daily injections of 3 cc. of breast milk showed changes identical with those of the 14 day animals given cow's milk.

The lungs of animals killed at one month were rubbery or crepitant, with irregular areas of collapse and emphysema. Extensive patchy granulomatous areas were seen on microscopic examination. Moderate fibrosis was present. Many alveolar walls were thickened. Occasional bronchi were completely packed with large foamy macrophages, often in solid-appearing clumps. Hemorrhage, collapse, and emphysema were prominent features.

One rabbit which received 3 cc. of breast milk on each of three consecutive days died in 39 days. Both lungs were rubbery and purplish-red, especially in dependent portions. Patchy areas of emphysema were present. The lungs floated in the fixative (4% formaldehyde). Microscopically many alveolar walls were thickened, and numerous areas of emphysema and collapse were found. A few small granulomas were found. Several bronchi and a few alveoli contained large vacuolated cells similar to the lipophages of mineral-oil pneumonia. Numerous macrophages and granulocytes were closely applied to the alveolar walls, most of which were so thickened that they did not appear functional.

Animals killed at six weeks showed extensive granulomas. Several of the granulomas were sharply outlined by a thin rim of fibrous tissue. The general appearance of these focal lesions was somewhat similar to that of the lesions of

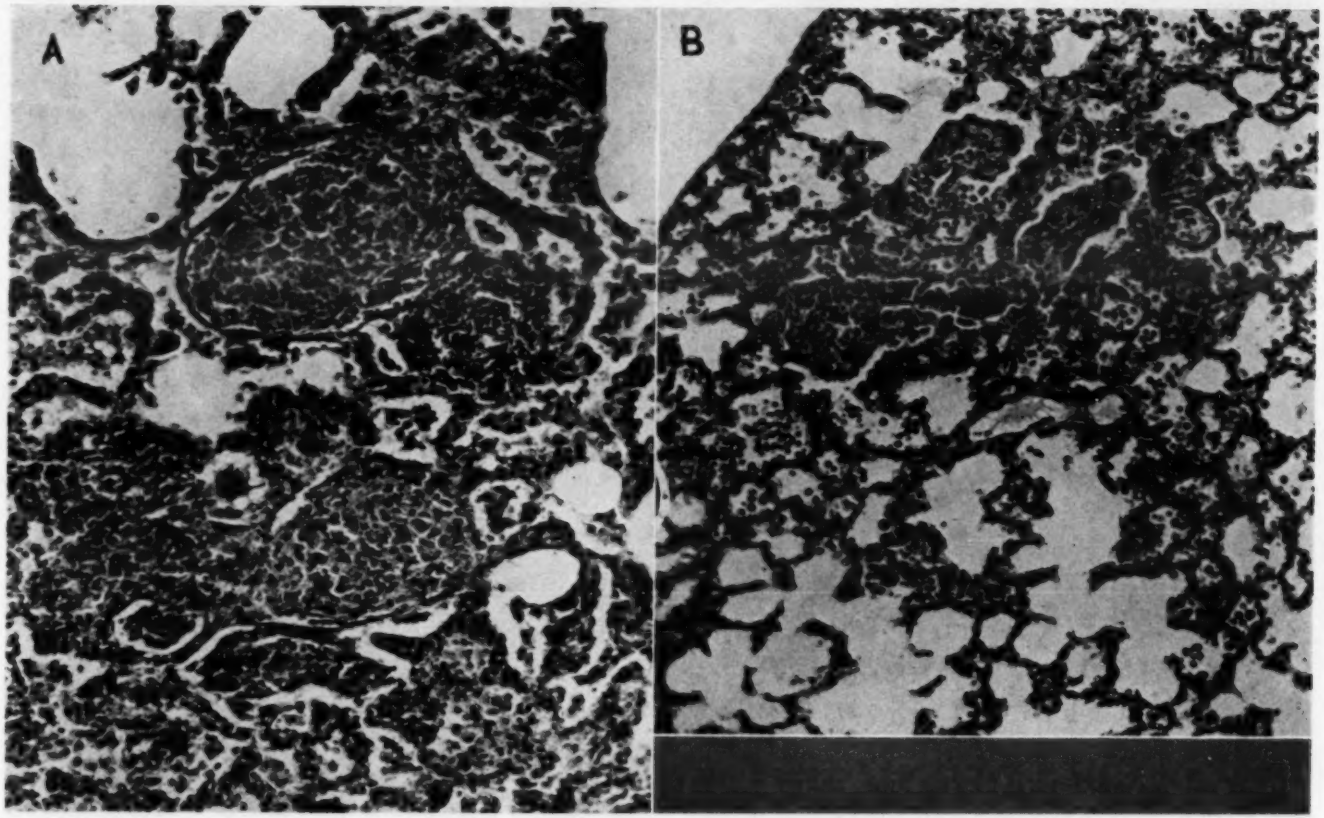


Fig. 7.—*A*, focal granulomas surrounded by mononuclear pneumonia in rabbit lung 23 days after initial injection; × 150. *B*, mononuclear pneumonia, granulomatous reaction, and emphysema six weeks after injection of milk into rabbit; × 150.

Boeck's sarcoid. Frequent foreign-body giant cells, some vacuolated and some containing pink-staining material, were found. Many alveolar walls were thickened. Early fibrosis, collapse, and emphysema were prominent features.

Animals killed two months after injection showed small or large granulomas with widespread, early fibrosis. Occasional giant cells and granulocytes were seen. Collapse and emphysema were again prominent.

One animal was given 5-cc. injections of feeding mixture 17 times in 22 days to determine whether repeated sublethal doses would eventually kill it and to see

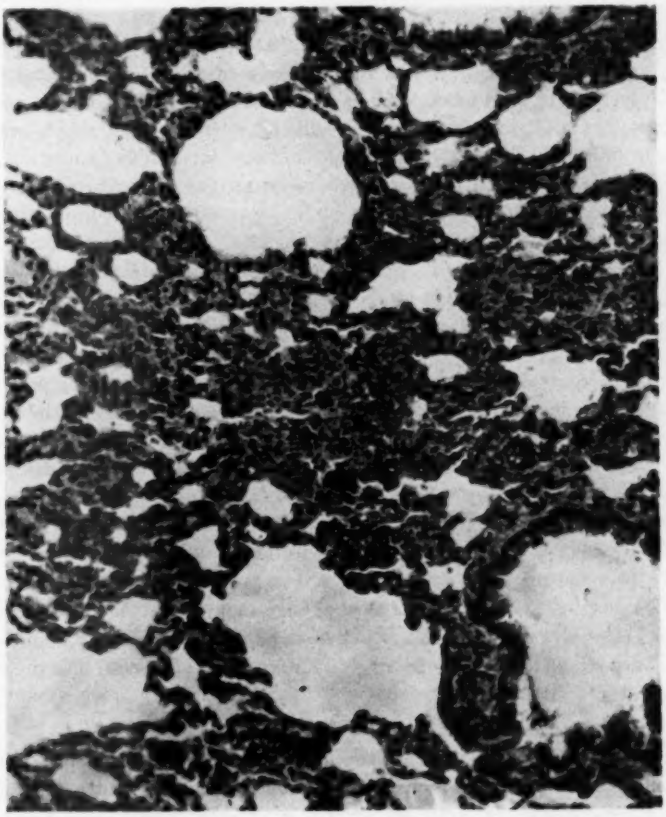


Fig. 8.—Early fibrosis and emphysema six weeks after injection of milk into rabbit; × 150.

whether the response was different from that in animals given only 1 to 3 injections. The rabbit died in 23 days of an extensive hemorrhagic pneumonia. Microscopically an extremely varied reaction was seen, with acute pneumonia and abscess formation and large and small granulomatous lesions. Many of the focal granulomas showed early peripheral fibrosis and resembled the lesions of Boeck's sarcoid or hyperplastic tuberculosis. Central accumulations of milk in certain granulomas occasionally resulted in a picture resembling that of miliary tuberculosis with necrosis. The granulomatous areas usually contained granulocytes in varying num-

bers. Numerous giant cells, often large and bizarrely shaped, were seen throughout the sections. The giant-cell reaction was much more prominent in this animal than in those given only one to three injections of milk. Many giant cells contained fat globules. One-half to two-thirds of the lung tissue was involved either by acute pneumonia or by chronic granulomatous lesions.

COMMENT

Some features of the reaction to milk injected intratracheally into rabbit lungs are similar to those seen after injection of oils or fat. This fact has been pointed out by Pinkerton,[5] who injected butter into two rabbits and cream into another and observed the similarity of the reaction to that produced by injection of cod liver oil. The general reaction, however, more closely resembles that caused by intratracheal injections of solid foods, such as peas, beans, and meat. Definite microscopic identification of milk in rabbit lungs is difficult even in early stages when large amounts of milk have been injected, and it is almost impossible to identify the milk after the first week or 10 days. The character of the reaction permits tentative microscopic diagnosis of the disease even in late stages, although differentiation from pneumonias caused by other foods is difficult.

The difficulty in making a definite pathologic diagnosis in this disease suggests the possibility that some sudden or relatively sudden unexplained deaths of infants may have been due to aspiration of milk. Fatal bronchopneumonias in infants, especially those with a mononuclear exudate, are classified by most pathologists as virus pneumonias. Gruenwald and Jacobi [6] in a discussion of mononuclear pneumonia in infants reviewed the literature on its etiology and suggested a viral infection as a good possibility. Hug [7] suggested that improper techniques in feeding premature infants resulted in repeated aspirations of small amounts of food. Hug's article, however, contained no postmortem observations. Sprunt [8] produced mononuclear pneumonias experimentally in a variety of ways, and he mentioned one instance of a mononuclear pneumonia produced by aspiration of milk.

While there is no doubt that mononuclear pneumonia can be produced by a variety of agents, the observations reported here suggest that many cases, especially in infants, may be caused by aspiration of milk. This possibility should at least be considered as an etiological factor in mononuclear pneumonias both in infants and in adults.

SUMMARY

The pathologic changes produced by aspiration of milk into human lungs have been described. The changes include acute pneumonia, abscess formation, focal and widespread granulomas, alveolar thickening, giant-cell reaction, and fibrosis. The reaction is usually mononuclear and granulomatous at the time of death. Giant-cell reaction is not constant and may be entirely absent.

5. Pinkerton, H.: The Reaction to Oils and Fats in the Lungs, Arch. Path. **5**:380, 1928.

6. Gruenwald, P., and Jacobi, M.: Mononuclear Pneumonia in Sudden Death or Rapidly Fatal Illness in Infants, J. Pediat. **39**:650, 1951.

7. Hug, E.: Das klinische Bild der interstitiellen plasmacellulären Pneumonie des Säuglings, Ann. pædiat. **159**:2, 1952.

8. Sprunt, D. H.: The Significance of Interstitial Mononuclear Pneumonia, South. M. J. **31**:362, 1938.

The tissue reaction produced by intratracheal injection of varying amounts of feeding mixture, human milk, or cow's milk into rabbit lungs has also been described. The tissue reactions evoked to the three types of milk are identical, and the changes produced are the same as those seen in human milk-aspiration pneumonia. However, the immediate reaction to large amounts (10 cc. or more) of feeding mixture is entirely different from the reaction to identical amounts of cow's milk or human milk. Relativcly small quantities of feeding mixture (10 to 20 cc.) often produced death from pulmonary edema in 1 to 11 minutes, while similar quantities of cow's milk or human milk rarely caused death.

A definite reaction to the milk occurred within a few minutes of the time of injection. Smears and sections of the milk in the bronchi showed numerous red blood cells, granulocytes, and macrophages. Occasional macrophages contained fat, apparently from the milk. Tissue sections showed early exudation of red blood cells, granulocytes, and macrophages in numbers greatly exceeding those in control animals.

Later reactions to injection of milk varied from acute pneumonia to focal and widespread granulomatous lesions with giant-cell reaction and fibrosis. Alveolar thickening was a prominent feature of the reaction. Emphysema was seen in connection with focal atelectasis or small or large granulomas.

Although some features of the disease both in human and in animal lungs resembled certain features of lipid pneumonia caused by liquid petrolatum or cod liver oil, the general reaction more closely resembled that seen in response to injection of solid foods. Because the response is to the milk protein as well as to the fat, the term milk-aspiration pneumonia more accurately describes this disease than terms such as lipid pneumonia caused by milk.

Difficulties in recognition of this disease both in animal and in human subjects are stressed. Microscopic studies of the human and animal material suggest that many of the mononuclear pneumonias occurring in infants may be caused by aspiration of milk. Knowledge of the microscopic appearance of milk in routine sections and understanding of the pathologic changes produced by milk in human and animal lungs should lead to more frequent recognition of this disease in human subjects, especially in infants and in old people. This in turn should emphasize the need of continuous instruction of nursing personnel on feeding methods and general nursing care designed to eliminate the hazards of aspiration pneumonia of any type.

DEVELOPMENTAL BRAIN METABOLISM

Effects of Cortisone, Anoxia Fluoroacetate, Radiation, Insulin, and Other Inhibitors on the Embryo, Newborn, and Adult

SAMUEL P. HICKS, M.D.

With the Technical Assistance of Alice A. Williams

BOSTON

THE CHANGING metabolism and cellular chemical processes that accompany mammalian development have much to do with how the organism responds at different ages to environmental stresses and noxious agents. The specific metabolic machinery of any given cell determines its vulnerability to injury; for example, one type of cell can function without oxygen for a period of time, while another succumbs almost at once. Furthermore, the same cell may show striking transitions in its metabolic patterns as it passes from embryonic to adult stages. The mechanisms of developmental malformations and disease processes that are peculiar to any given period during the life of the organism are therefore closely bound with these changes.

Experiments in our laboratory have been carried out in which a variety of enzyme inhibitors and other agents that interfere with various stages of metabolism have been given to living animals and pathologic examinations have then been made to determine which cells, tissues, or components of organs were specifically attacked. The basic assumption in this type of experiment is that the cell most susceptible to the agent will be damaged because its metabolic machinery is more vulnerable than that of a neighboring different kind of cell.

There are three established methods of studying enzymatic processes in cells and tissues. One is to measure the chemical processes in a slice or ground-up sample of a given organ isolated from the living animal. Another is to demonstrate by histochemical "stains" the enzymatic processes in an isolated piece of tissue. The third, a physiologic method, is to observe in an organ changes in function and chemical behavior that result from administration of various agents to living animals or humans. The first method often deals with tissues either in a disintegrated state or quite apart from the total organism. The third sometimes reveals what is often the most important aspect, change in function; but this observation alone sometimes is misleading and the organ most severely damaged may escape notice.

The present study employs a fourth method, which is essentially a pathologic one. It determines what cells or tissues are irreversibly damaged by the particular

From the Department of Pathology and the Cancer Research Institute of the New England Deaconess Hospital and the Departments of Pathology, Harvard Cancer Commission, and Harvard Medical School.

This work was supported by the United Cerebral Palsy Association, United States Public Health Service Grant C-1042, and Atomic Energy Commission Contracts AT(30-1)-699 and AT(30-1)901.

metabolism-inhibiting procedure, but it does so by interpreting morphologic changes in the light of the other three viewpoints and to some extent incorporating their methods.

The administration of drugs, enzyme inhibitors, antimetabolites, and other agents to living animals with the aim of having the agent pick out one enzyme system in one type of cell and not affect any others directly or indirectly is a complex problem and probably impossible of pure achievement, because all cells probably utilize certain basic metabolic pathways,[1] the differences among cells being in the size of the pathways, the presence of alternate ones, and the possession of metabolic paths to synthesize certain products or carry out certain functions that other cells virtually lack. The prime advantage of this method of giving drugs to animals and determining what tissues are selectively damaged is that the positive product of the study is an animal with a lesion. The disadvantages are similar to those found in physiologic and biochemical studies. They are the overlapping effects of some inhibitors on more than one cell or enzyme, together with uncertainty as to whether the agent acts in the cell or at its surface, how long it actually does act and how fast it permeates the cell, whether accumulation of the agent in one kind of cell has any relation to its effects on that cell, and finally what secondary effects the agent may have by acting on the circulatory, respiratory, or other system.

These disadvantages can be partly overcome by interpreting the pathologic results in the light of reasonably well-established biochemical, histochemical, physiologic, and clinical observations. They can also be partly obviated by employing several different agents that are believed to have a similar biochemical action in vitro and seeing whether their pathologic effects are similar. To some extent the differing permeability of different tissues and cells to a given agent can be circumvented by pressing its effect to a limit which when exceeded kills the total organism.

A number of agents that inhibit metabolism have already been applied to adult, embryo, and newborn animals.[2] Past experiments have considered the pathologic effects on all tissues, with prime emphasis on the nervous system. Adult rats were treated with acutely sublethal doses of (among other agents) azide, cyanide, malononitrile, insulin (hypoglycemia), and nitrogen (asphyxia). These five agents singled out for destruction the neurons of the cerebral cortex, striatum, hippocampus, and substantia nigra, but the patterns after use of each agent had significant differences in some respects. In addition to these differences was the most interesting fact that only cyanide, azide, and malononitrile damaged white matter—myelinated fibers of the striatum, corpus callosum, and sometimes optic nerves and cerebellum. The gray-matter patterns were attributed to the immediate dependence

1. Buchanan, J. M., and Hastings, A. B.: Use of Isotopically Marked Carbon in Study of Intermediate Metabolism, Physiol. Rev. **26**:120, 1946.

2. Hicks, S. P.: Brain Metabolism in Vivo: I. Distribution of Lesions Caused by Cyanide Poisoning, Insulin Hypoglycemia, Asphyxia in Nitrogen and Fluoroacetate Poisoning in Rats, Arch. Path. **49**:111, 1950; Brain Metabolism in Vivo: II. The Distribution of Lesions Caused by Azide, Malononitrile, Plasmocid and Dinitrophenol Poisoning in Rats, ibid. **50**:545, 1950; Some Effects of Ionizing Radiation and Metabolic Inhibition on the Developing Mammalian Nervous System, J. Pediat. **40**:489, 1952; Effects of Ionizing Radiations on the Adult and Embryonic Nervous System, in Toxic and Metabolic Diseases of the Nervous System, A. Res. Nerv. & Ment. Dis., Proc., to be published.

of the brain, generally speaking, upon the aerobic oxidation of glucose and the high metabolic rate of gray matter. The additional action on white matter was attributed to the fact that although cerebral white matter has a considerably lower metabolic rate than gray it also has a very small quota of cytochrome oxidase, for which these three agents have an affinity. Thus cytochrome oxidase would seem to constitute an enzymatic bottleneck for cerebral white matter. If only one of these agents selectively damaged white matter, the biochemical mechanism would be in doubt. When three agents that are chemically unlike, and have different durations of action, do so, there is strong evidence that a specific enzymatic inhibition is the mechanism. In this connection it is notable that these agents specifically damaged an appendage of a nerve cell without killing the cell body. The associated necrosis of oligodendroglia cells in damaged areas may have an important bearing on demyelinating diseases; the relation of the oligodendroglia to the myelin sheath is still poorly understood.

Other experiments have shown equally interesting results, though the interpretation of the mechanisms involved are neither simple nor completely understood. Fluoroacetate consistently caused acute necrosis of cardiac muscle and of the maturing elements [3] in the testicular tubular epithelium. It produced mild cardiac arrhythmias and severe spastic convulsive episodes; yet the irreversible damage to the brain was slight and that to the heart often severe. Fluoroacetate acts in the Krebs cycle, possibly at more than one site, to block certain phases of citrate and acetate metabolism. The special vulnerability of the heart may be related to its dependence upon two and three carbon fragments as well as glucose as substrates. Blocking of acetate utilization and suppression of part of the Krebs cycle, the energy of which is coupled to the formation of energy-rich phosphate bonds (essential to glucose catabolism), could strike a double blow at heart muscle. Still another drug, whose action is unknown biochemically, is "plasmocid," an 8-aminoquinoline derivative. This compound causes severe widespread necrosis of skeletal muscle and cardiac muscle and less extensively necrotizes Gasserian ganglion neurons.

These and many other agents were also given to pregnant and newborn animals. Ionizing radiations selectively necrotized neuroblasts in embryo and newborn animals, yet spared neurectoderm and neurons of the newborn and adult, unless the dosage was carried to extraordinary levels. This necrosis of neuroblasts is one of the most specific destructive biologic effects that can be produced experimentally. Forty roentgens (200 kv., 3 mm. Al, 10 ma., 50 cm.) selectively destroyed some neuroblasts when a pregnant mouse or rat was given total body exposure; yet neurectoderm withstood at least 10 times this dose and adult neurons were rarely or not at all destroyed by 1,500 r and were rarely damaged by 20,000 r. This differential effect as the nerve cell passes from embryonic to adult life is a good example of changing developmental metabolism. The finding has already been used in two fields of investigation: 1. The selective destruction of neuroblasts in successive stages of development has led to the production of a whole chronologic series of brain malformations from anencephaly through cerebral anomalies to cerebellar defects; these malformations are reproducible and predictable because they depend on which neuroblast building blocks are present at the specific moment of develop-

3. Principally stages from spermatocyte to spermatid.

ment. 2. This radiation effect can also be used as a tool with which to study developmental metabolism and the chemical mechanisms of acute radiation reaction; these are of principal concern here.

Some experiments in the second field have already been carried out. Biochemical studies by others had shown that ionizing radiations may produce their effects chiefly by causing the formation of short-lived oxidizing compounds in the cell water, which in turn disturb the oxidation reduction equilibrium of enzymes with easily oxidizable sulfhydryl prosthetic groups [4] and possibly also upset the ratio of reduced to oxidized glutathione.[5] (Such enzymes have been associated with nucleic acid and protein synthesis in certain forms of cell growth, especially differential growth.) To test these in vitro observations a number of sulfhydryl reagents and radiomimetic drugs were given to show whether chemicals that attack sulfhydryl compounds, or otherwise imitate biochemical effects of radiation, would indeed imitate radiation in the live animal. Nitrogen mustard (methyl bis- or tris-[β-chloroethyl] amine hydrochloride) and triethylene melamine were given to pregnant and newborn animals. Neuroblasts in the fetuses and newborn were extensively necrotized in a pattern similar to that seen following use of ionizing radiations. These agents were a little less selective than radiation, and the "margin of safety" was smaller between a dose that selectively damaged neuroblasts and one that would also damage other fetal tissues, such as cartilage, or maternal bone marrow. Chlormercuribenzoate, oxophenarsine hydrochloride (mapharsen®), iodosobenzoate, and porphyrindin, which are reagents for sulfhydryl groups in biochemical determinations, were also given to pregnant and newborn animals. To a degree less striking than nitrogen mustard and triethylene melamine these compounds also caused necrosis of neuroblasts. Iodoacetate, which is usually called a sulfhydryl reagent and which inhibits the enzyme phosphoglyceraldehyde dehydrogenase, did not behave like any other sulfhydryl reagent and failed to damage neuroblasts even when pressed to the lethal zone. This observation will be significant in the experiments that are to follow.

These compounds were given, as in the foregoing experiments, in doses just sublethal to the mother, the fetus, or the newborn animal, as the experiment might dictate. The lethal dose was determined first by trial and error; or if in a litter of fetuses some were dead, it was established that the experimental maximal-tolerated sublethal dose had been administered. Essentially, then, the acute $L.D._{50}$ was being used in most of these experiments. If under these circumstances one tissue was singled out and others spared, then there existed a metabolic weak spot or bottleneck for that agent. If the total animal always died instead of revealing a vulnerable tissue, then no experimentally measurable differential weakness existed.

Thus this approach to metabolism through pathologic methods demonstrates primarily differences in metabolic pathways and not so much their absolute existence. The significance of the failure of a drug to act selectively is simply that an appreciable difference does not exist, assuming that in the experiment the lethal dose for the whole animal has been closely approached. For example, a dose of

4. Barron, E. S. G.: Thiol Groups of Biologic Importance, Advances Enzymol. **11**:201, 1951.

5. Danielli, J. F., and Davies, J. T.: Reactions at Interfaces in Relation to Biological Problems: III. Partition of SH Groups Between Surface and Bulk Phases, Advances Enzymol. **11**:47, 1951.

cyanide that quickly kills all of a group of rats reveals little. If a dose is given that kills half the rats, there will be pathologic evidence among some of or all the survivors that certain cells are specifically damaged irreversibly. Of course with an agent like radiation, the lethal dose for the total animal need not be approached simply to demonstrate a selective action. Many agents lie between these extremes, and, other factors being equal, the degree of specificity is proportional to the "margin of safety" between selective action and death of the animal.

From these experiments and others that have been reported elsewhere in detail,[2] some beginning metabolic characterizations of the nerve cell as it develops have been made. Neurectoderm was not damaged in experiments in which anoxia, hypoglycemia, or other agents that interrupted aerobic oxidation of glucose were involved; nor was it damaged by any of the radiomimetic drugs, sulfhydryl reagents, "aminopterin" (4-aminopteroylglutamic acid), or radiation. In contrast, the neuroblast was sensitive to the radiation-sulfhydryl group of agents but resisted interruption of its aerobic processes. This observation does not imply that neuroblasts do not ultimately depend on oxygen and perhaps glucose; it means that, as compared with the adult neuron (which depends almost exclusively on aerobic catabolism of glucose), the neuroblast is not immediately dependent upon these pathways to function. This observation is exemplified by the fact that the neuroblast was not selectively vulnerable to hypoglycemia—the mother or sometimes the total fetus died before the neuroblasts were harmed under the conditions of experimental insulin hypoglycemia.

These experiments, then, demonstrated some characteristics of embryonic nervous tissue and adult tissue. Little has been known from the pathologic standpoint about the metabolism of the neonatal nervous system, although classic studies by Himwich and his associates [6] have shown that the newborn rat is extraordinarily resistant to anoxia and has available two pathways for glycolysis to accomplish resistance. Infant rats lived nearly an hour in nitrogen, but this survival was halted by iodoacetate and by fluoride, which block anaerobic glycolysis at the phosphoglyceraldehyde dehydrogenase and enolase levels. These agents did not interfere with aerobic glycolysis, for the animals survived in air with these drugs. No data were available on whether the anoxia or the drugs did any selective damage to brain or other organs, undetected by usual physiologic observations, and the source of anaerobic energy was assumed to be glycogen, but from origins undetermined.

It is the purpose of this paper to report on experiments which have extended this study of differences of metabolism amongst cells of the body, especially in the newborn and as it relates to development. Three types of experiments have been carried out. The first has been to investigate the nature of the resistance of the newborn to anoxia, to demonstrate by pathologic methods whether prolonged neonatal asphyxia does any permanent damage, and to find out what is the source of anaerobic energy during this survival. The second has been to extend the study of sulfhydryl reagents in their effects on neuroblasts; these agents are difficult to apply experimentally to live animals, and negative results are frequent because of the "low margin of safety." The sulfhydryl enzymes are important in relation to radiation effects and growth processes and are in need of continued investigation.

6. Himwich, H. E.: Brain Metabolism and Cerebral Disorders, Baltimore, Williams & Wilkins Company, 1951.

The third type of experiments has been to investigate the effects of certain steroid hormones and related substances on the developing and adult nervous system. The rationale for incorporating these into the present study is evident. Cortisone has been judged to act on a group of sulfhydryl enzymes concerned with cell growth and differentiation.[7] Steroid hormones have been shown to affect cerebral metabolism both in vitro and clinically,[8] and, contrary to some popular concepts, there is reason for viewing them as a group with certain common actions and also as highly function-specific agents. Several steroids, including cortisone, appear to have a braking action on glucose metabolism, possibly at the hexokinase level. A third reason for incorporating steroid experiments into this study is that preliminary experiments had shown that cortisone (and corticotropin) caused necrosis of neuroblasts.

A fourth objective has been to integrate past and present experiments into something of a whole by tabulating the responses of the nerve cell, as it develops, to various groups of metabolic reagents and then constructing a metabolic model based on the tabulation. Crystallizing experimental data into models has the useful effect of integrating the work into more than just tabulations, so that defects of reasoning about them and new lines of approach are defined.

MATERIALS AND METHODS

In general the chief experimental method has been to administer the particular form of metabolic inhibition to adult, newborn, and pregnant rats and mice and to study the animals pathologically about 24 hours later, when lesions have had time to become visible. (Late developmental effects will be considered elsewhere and referred to only briefly here.) In some experiments slightly longer intervals were used. In other experiments, involving asphyxia of newborn animals in nitrogen, the duration of survival, not a pathologic lesion, was the objective. In still others, histochemical glycogen stains were done at various intervals after an agent was administered.

In the pathologic studies the adult and newborn animals were killed by section of the upper thoracic spine and complete autopsies done. Fetuses were removed and, according to their size, fixed whole or in multiple blocks. The routine pathologic examination for adult animals consisted of multiple or serial sections of the whole brain; thoracic spine with cord, muscles, and usually ganglia; eyes; Gasserian ganglion; pituitary; one to three sections of tongue; masseter muscle with salivary gland, and sometimes lymph node, neck organs (larynx, esophagus, thyroid, and parathyroids), external ear (for skin), thymus, heart, lung, liver, spleen, pancreas, adrenal, kidney, abdominal muscle, diaphragm, testes with epididymis and seminal vesicle, hamstring muscles, and sciatic nerve. The thoracic aorta and some other large vessels were usually included. The pituitary was sometimes lost for technical reasons, and the parathyroids are not always evident in routine sections. Usually two large blocks of the heart were made.

7. Anderson, G. E., and others: Sulfhydryl Inhibition as a Mechanism in the Effects of ACTH and Cortisone, Proc. Soc. Exper. Biol. & Med. **76**:825, 1951. Stock, C. C.; Karnofsky, D. A., and Suguira, K.: Studies of Steroids for Inhibition of Normal and Abnormal Growth of Experimental Animals, in White, A., editor: Symposium on Steroids in Experimental and Clinical Practice, Philadelphia, The Blakiston Company, 1951.

8. Gordan, G. S.; Bentinck, R. C., and Eisenberg, E.: Influence of Steroids on Cerebral Metabolism, Ann. New York Acad. Sc. **54**:575, 1951.

In previous experiments, especially when cyanide, insulin, asphyxia, and so on, were used in adult animals, serial sections were often made of the whole brain. The dead neurons were plotted by the numbered areas of the cortex as outlined by Krieg and the nuclei of the brain stem as shown by Gurdjian. This procedure led to an enormous body of data, especially in regard to cortical areas. Though general differences of distribution of lesions in these brains were of interest, the significance of slight variations of cortical lesions by areas and cell layers was not established, and the mapping procedure was discontinued. Thereafter the brain was divided into about eight frontally sectioned blocks in rats and about five in mice. From each, three ribbons were sectioned as a rule, though in some instances several levels or serial sections were examined.

In the case of newborn animals and fetuses several procedures were used. Newborn animals in all experiments were literally a few minutes to 3½ days old. Fetal experiments were carried out for convenience [2] on mothers about 14 to 21 days pregnant; the youngest animals were fixed whole, and ribbons were cut at about three to five levels. Occasionally serial or multiple sections were made. In older fetuses and newborn animals the head was made into four or five frontal blocks and the remainder of the animal into similar blocks about 2 to 3 mm. thick. Each block was sectioned one to several times, so that a fair sampling was obtained. Serial sections were rarely made, because at present the experiments are aimed at determining acute damage to regions rather than to discrete nuclei or individual small groups of cells. (In other experiments, to be reported elsewhere, in which the late effects on development were the aim of the study, serial sections were made.) From pregnant animals, usually four or five fetuses were sampled, occasionally more. In all litters several placentas were studied histologically.

The method for preparing tissues for routine examination was to immerse them in modified Bouin's fixing fluid made up of 10% formalin with 5% acetic acid and saturated with picric acid. Occasionally neutral 10% formalin was used. Embedding was in paraffin. For glycogen staining, the tissue, cut as described, was immersed overnight in cold (about 40 F.) Rossman's picric-acid-formalin-alcohol fluid and then immediately carried through alcohols and embedded in paraffin. Hematoxylin and eosin was the stain of choice for revealing dead cells, especially when they were few and widely scattered in the brain. Nicolle's carbol-thionine was sometimes useful. Bodian's strong silver protein stain for nerve fibers, Mallory's phosphotungstic-acid hematoxylin, and a modified Masson trichrome stain were sometimes used. McManus' periodic acid-Schiff reagent method was used for glycogen, usually on untreated slides but occasionally after saliva or diastase digestion.

The criteria for damage to the nervous system were established upon a basis of previous experience with several hundred animals and are appropriately detailed here as part of the method. A dead neuron such as that which results from asphyxia, cyanide, or hypoglycemia becomes reduced markedly in cytoplasmic and in nuclear volume. Within 16 to 24 hours this acute change is maximal. The nerve cell may be one-third to one-sixth the diameter of its living neighbor, with eosinophilic cytoplasm and similarly reduced nucleus. The cell and nuclear outlines are usually indistinct, and the nucleus becomes homogeneously basophilic or, sometimes, beaded and coagulated. The dead neuroblast becomes similarly reduced in size, but because it has little cytoplasm this component is not a conspicuous feature. What cytoplasm

is recognizable either becomes eosinophilic or does not stain. The nucleus shrivels to about one-half to one-fourth its original diameter and becomes a coagulated, often lobulated or beaded mass of densely basophilic material. After use of radiation or nitrogen mustard this condition is well developed in as little as four hours. The neuroblasts most vulnerable to radiation, nitrogen mustard, and sulfhydryl reagents have been those in the medial striate mass just lateral to the ventricles and at the angles where these abut the corpus callosum or its future situation. After cortisone, and in the newborn after any effective neuroblastocidal agent, the outer-cortex neuroblasts are especially susceptible. In animals old enough to have a corpus callosum, cells in it that are developing into oligodendroglia at the ventricular angles and lateralward are similarly sensitive to these agents.

In fetal animals, when substantial numbers of neuroblasts are necrotized, phagocytosis begins in 6 hours, and macrophages filled with cell detritus may be conspicuous in 24 hours. In adult animals, scattered dead neurons excite no phagocytic or leucocytic response. Under these circumstances they literally fade away without a residual trace in a few days to a week or so. When many are damaged or severe white-matter damage occurs, phagocytes play a fairly prominent role, becoming conspicuous in 24 to 48 hours.

Any damage to neurons is usually accompanied by a relatively reversible phenomenon in the vascular and interstitial cells. This is characterized by enlargement, sometimes elongation, of the nuclei of endothelial cells and to some extent by swelling of the cytoplasm. Nuclei of oligodendroglia and microglia cells become hyperchromatic, as do some of those in endothelial cells. Occasionally mitoses occur in microglia. There is usually a slight increase in the "artifactual" space around the capillaries, and this has been notable after a number of agents in the adult striatum (caudate, putamen, and globus pallidus masses). This reaction, which develops in less than 24 hours and subsides or persists according to the degree of parenchymal damage, is so nearly a constant finding that it became a guide, especially in the cortex and striatum (where damage was so often found in adults [2]), to reveal scattered necrotic neurons, which could easily be overlooked unless they were intensely searched for. In previous experiments these changes were also seen in animals in whom a careful search revealed no dead neurons yet whose mates in the experiment did show neuronal damage. The changes therefore indicated that an effect short of necrotizing neurons had occurred, but because it could not accurately be determined how far short, such animals were not "counted" as showing a positive effect. Nuclear hyperchromicity of vascular and interstitial cells in very mild form is hard to tell from normal variations, but it does serve as an indication in brains that are routinely examined. Spontaneous necrosis of neurons never occurred in control rats and mice.

The animals used were white rats from the Harvard University Biological Laboratory, a strain originally from the Wistar Institute and inbred for about 20 years. Mice were chiefly of two strains, obtained from the Jackson Memorial Laboratory at Bar Harbor, Maine: BAF_1, and ABC. These first-generation hybrids were allowed to inbreed for experiments in which pregnant and newborn animals were required. A strain of mice, yellow crossed with black-and-white, obtained from the Oak Ridge National Biological Laboratory, was used in asphyxia and cortisone experiments. Variability of response within a given strain in these

experiments was greater than any that could be detected between strains. Differences between rats and mice in these acute experiments have not yet been detected, but species differences will doubtless arise, especially as more steroid experiments are carried out.

ANOXIA EXPERIMENTS IN NEWBORN ANIMALS

(*a*) *Simple Anoxia.*—Sixty-two newborn mice and rats were immersed in nitrogen gas and their survival time noted. Animals starved 24 hours from birth, or 2 days old and starved 24 hours, as well as nursing animals, were used. The gas used was 99.97% nitrogen with 0.03% oxygen. The immersion was carried out in a glass flask through which nitrogen flowed at 2 to 6 liters a minute. The gas was bubbled through water en route to the flask. The experiment was done at room temperature, about 24 to 29 C. (Effects of other temperatures were not investigated.) Fourteen animals were removed alive at intervals of 30 to 50 minutes for pathologic examinations 24 hours later and 10 others similarly for immediate glycogen studies. In addition, three groups of animals (four in each group) 1 week, 10 days, and 16 days old were similarly tested for survival only in nitrogen.

(*b*) *Anoxia with Iodoacetate or Fluoride.*—Newborn rats and mice were subjected to anoxia, as described, after subcutaneous injections of solutions of sodium iodoacetate (10 to 100 mg. per kilogram, five animals) or sodium fluoride (50 to 150 mg. per kilogram, five animals, mice only). These doses were either sublethal or not lethal for several hours for an animal breathing air, and they were done simply to confirm Himwich's experiments, which showed that these drugs would shorten anaerobic survival. Anoxia was induced in different animals about 15 minutes, 1 hour, 2 hours, and 3 hours after the drugs were given to see whether the drugs varied in effects over this period. Since iodoacetate completely abolished the normal newborn animal's resistance to anoxia, by trial and error a dose was contrived (about 2 to 5 mg. per kilogram) small enough to permit the animal to survive about 20 minutes in nitrogen. Three additional animals were treated this way, then kept with the mother overnight, killed, and studied pathologically to see what cells if any were damaged by partially blocking both the aerobic and the anaerobic glycolytic pathways.

(*c*) *Anoxia with Sulfhydryl Reagents.*—Rats and mice were treated with sodium chlormercuribenzoate (50 mg. per kilogram, seven animals) and oxophenarsine hydrochloride (20 mg. per kilogram, five animals) subcutaneously, which are sublethal doses that would kill in a few hours or just fail to kill. At intervals of 30 minutes, 1½ hours, and 4 hours after receiving the drug different animals were immersed in nitrogen as in foregoing experiments to test whether these agents could interrupt anaerobic survival. These agents were used for comparison of their action with that of iodoacetate, which, as already noted, seemed to be a unique sulfhydryl reagent acting only at one enzymatic site. These agents did not shorten survival in nitrogen, and all the animals were taken alive after 35 to 50 minutes of asphyxia, returned to the mother, and studied pathologically the next day.

(*d*) *Anoxia with Fluoroacetate.*—Rats and mice were tested for the possible blocking action of this agent against anaerobic metabolism, as was done with the sulfhydryl reagents. Sodium fluoroacetate (2 to 3 mg. per kilogram, 17 animals) was given subcutaneously one-half to three hours before testing. This drug inter-

rupts aerobic processes in the Krebs cycle, but if it could suppress the formation of anaerobic energy-rich stores (phosphate bonds) it might hinder later anaerobic survival.

(*e*) *Anoxia with Cortisone or Corticotropin.*—Experiments similar to those just noted were carried out with subcutaneous injections of cortisone or corticotropin. Doses were from 0.25 to 1 mg. of cortisone (Armour's preparation, 25 mg. per cubic centimeter) or ¼ to 1 unit of adrenocorticotropic hormone (Armour's acthar,* 5 mg. or units per cubic centimeter). Twenty animals were tested for interference with anaerobic survival 3 hours, 6 hours, and 24 hours after receiving cortisone. Twelve animals were tested in nitrogen 6 and 24 hours after corticotropin; three were removed after 9 to 22 minutes and 6 after 35 to 45 minutes of asphyxia and studied pathologically 24 hours later. Four cortisone-treated animals were taken alive after 40 to 55 minutes for immediate glycogen studies, and six others were examined pathologically 24 hours after being asphyxiated for 40 to 55 minutes. These agents were used because of their alleged dual action on sulfhydryl enzymes and glucose metabolism.

(*f*) *Anoxia with Insulin Hypoglycemia.*—Experiments similar to the foregoing tested the effect of insulin hypoglycemia on 14 newborn animals. About four hours after receiving 2 to 4 units of insulin subcutaneously they were asphyxiated and the survival time noted. If glucose were the principal substrate used during anoxia, survival should be shortened. Only one animal survived 30 minutes, and it was studied pathologically 24 hours later. Two of those dead in less than 20 minutes were studied for glycogen.

(*g*) *Anoxia with Ionizing Radiation.*—Newborn animals were irradiated with about 150 r in nitrogen. (Factors: General Electric Maximar therapy unit, 200 kv., 10 ma., 3 mm. inherent Al filter, 25 cm. target distance, about 50 r a minute.) This amount of radiation consistently damages the neuroblasts to a severe degree, but in hundreds of animals it did not necrotize neonatal or adult neurons. Radiation was given to animals in nitrogen after 5 minutes and 15 minutes of asphyxia (four animals in each group). The purpose was twofold: (1) to see whether anoxia protected neuroblasts from the well-known destructive effects of radiation, as it is said to protect animals from other effects of radiation; (2) to see whether radiation would injure other cells which during anoxia would have to shift from aerobic glycolysis and anaerobic glycolysis, in which the sulfhydryl enzyme, phosphoglyceraldehyde dehydrogenase, functions.

HYPOGLYCEMIA EXPERIMENTS IN NEWBORN ANIMALS

Insulin was given parenterally to newborn rats and mice 1 to 3 days old, and they were studied in different ways. Three litters (four each), starved from birth and never having nursed, were given the drug and then returned to the mother. Eleven nursing animals from a few hours to 3 days old were similarly treated. All were prepared for pathologic study 24 hours later. Two newborn litters, starved from birth, were given insulin at once and not returned to the mother. They died in 16 to 24 hours. Three, moribund, were examined pathologically. Four rats 2 days old, two starved 24 hours and two feeding, were given insulin and 4 hours later killed for blood sugar determinations, simply to confirm the observations of others.

Insulin was crystalline, 80 units per cubic centimeter; mice (1 to 3 gm.) and rats (8 to 10 gm.) were given about 2 and 4 units subcutaneously. (Animals were returned to the mother eight hours after receiving insulin.)

CORTISONE EXPERIMENTS

(*a*) *Pregnant Animals.*—Pregnant mice were given single subcutaneous doses of cortisone. Eight, pregnant from about 14 days to term, are dealt with here, although for other purposes animals with timed pregnancies [9] of 1 to 10 days were used and observations that are pertinent will be mentioned. Three animals received 1 mg. subcutaneously twice daily for four days, and five animals received 1 mg. once. They were killed 24 hours after the dose, and fetuses were removed. (Cortisone given in the first 12 days of gestation, although it did not apparently damage the embryos, shortened the pregnancy to 18 days in some instances; 20 to 21 days is usual.)

(*b*) *Newborn Animals.*—Nineteen litters of newborn mice and one litter of rats were given a single subcutaneous dose of cortisone (0.25 to 0.5 mg.) except that four received three daily doses for three days. These litters had four to eight animals; two or more animals from each were killed for pathologic or whole-skeleton studies on the day after the drug was given, and others were killed at frequent intervals thereafter. The purpose of this procedure was to see the acute immediate effects in the brain and skeleton and (for another study) to follow developmental changes.

(*c*) *Other Adult Animals.*—Cortisone was given subcutaneously in single or repeated daily doses up to four days. Four female mice received 2.5 mg. (single dose), and two males and two females, 2.5 mg. twice daily for three days.

CORTICOTROPIN EXPERIMENTS

Pregnant (latter third) and newborn mice were given corticotropin subcutaneously. Four pregnant animals received single doses of 5 units (5 mg.) and were killed 24 hours later, and both mother and young were studied pathologically. Four litters of newborn mice were given 0.5 mg. subcutaneously and studied pathologically 24 hours later. Four adult male rats received 12 mg. intraperitoneally and were studied pathologically four days later. Two male and two female mice received 5 mg. subcutaneously and were studied 24 hours later. One male rate received 5 mg. intraperitoneally and was studied two days later. The time intervals were varied to see whether the lesions varied as a result.

SULFHYDRYL-REAGENT EXPERIMENTS [2]

Previous experiments showed that chlormercuribenzoate, oxophenarsine hydrochloride, and iodosobenzoate, which are chemically classed as sulfhydryl reagents—chemicals which combine with SH groups—would damage neuroblasts in a pattern somewhat similar to that of radiation. Only iodoacetate did not do this, and, though it is often called a sulfhydryl reagent, it so differed from the others in behavior that throughout these experiments it was considered in a class by itself. Some

9. Gestation counted from the day on which spermatozoa could be demonstrated in the animal's vagina.

combinations of sulfhydryl reagents in appropriate fractional doses damaged neuroblasts, indicating that they were "synergistic" and had a common effect. As with the individual agents, the margin of safety was narrow, and a fair number of deaths or failures to get specific neuroblast damage occurred. Therefore the additive effect of fractional doses of sulfhydryl reagents was investigated further. Three pregnant mice were given one-fifth the corresponding near-lethal doses of oxophenarsine hydrochloride, iodosobenzoate, iodoacetate, chlormercuribenzoate, and porphyrindin. One animal was given one-fourth doses of all these agents except porphyrindin. One animal was given half-doses of porphyrindin and iodosobenzoate and another half-doses of oxophenarsine hydrochloride and iodosobenzoate. Four animals received half-doses of iodoacetate and oxophenarsine hydrochloride. Another animal got iodosobenzoate, chlormercuribenzoate, and oxophenarsine hydrochloride, each in one-third doses. (The approximate $L.D._{50}$ doses have been detailed elsewhere. They are oxophenarsine hydrochloride 20 mg., chlormercuribenzoate 50 mg., and iodosobenzoate 150 mg. The drugs, being incompatible when mixed, were given subcutaneously nearly simultaneously. Porphyrindin [200 mg.] given to one pregnant mouse produced some neuroblastic necrosis in the fetuses.)

FLUOROACETATE EXPERIMENTS

Fluoroacetate had damaged occasional neurons in adults,[2] and neuroblasts in some fetuses in previous experiments (three litters), although it failed to do so in several.[7] Also, in newborn animals single doses (1.5 to 2 mg. per kilogram in a single dose was close to lethal) caused some necrosis of cortical neuroblasts and little or none of the more deeply situated ones, but occasionally necrotized some of the cortical and brain-stem neurons.

For these morphologic and certain other reasons indicated later, this drug was given in three and five successive divided doses to a number of newborn rats and mice over a period of two to three hours to intensify the effect. This procedure was followed purposely, even though it increased the problem of comparing the "acute" effects of a number of agents whose duration of action can only be surmised by observation and never accurately known in vivo. Six groups of two or three rats were treated, and they were studied pathologically 24 hours later.

IODOACETATE AND FLUORIDE EXPERIMENTS

Five newborn mice were given 10 mg. per kilogram of iodoacetate subcutaneously. One was placed in nitrogen and died in less than 15 minutes. Three others lived 24 hours, to be studied pathologically, and the fourth died in 24 hours. Six newborn rats survived 50 or 90 mg. per kilogram of sodium fluoride, 90 mg. being virtually always lethal, and were studied pathologically after 24 hours.

EXPERIMENTS WITH OTHER STEROID HORMONES AND RELATED SUBSTANCES

A number of steroid hormones and related substances have been administered to adult, pregnant, and newborn rats and mice for the same dual purpose for which the cortisone and corticotropin were given, namely, study of the acute effects on the developing nervous system and study of late developmental alterations of growth of the skeleton and nervous system. The series has been only partly carried out,

but since steroids in large doses may have common actions on growth and on the nervous system that may ultimately reveal their mechanisms of action, the experiments will be briefly mentioned. All adults were studied pathologically 24 to 48 hours after administration of the drug. Among adult animals, diethylstilbestrol (stilbestrol*) was given subcutaneously to seven male mice, two nonpregnant mice, two pregnant mice, and one male rat in 3 to 12 mg. doses. Two litters of newborn mice (12) were given 0.015 mg., and four were examined pathologically within 48 hours. The drug was given in sesame oil except to the two female mice, to which an aqueous suspension was given. Estrone (wynestrone*) was given to two nonpregnant and two pregnant mice and two male mice intraperitoneally or subcutaneously. (With either route, no effects on adult or embryonic nervous systems occurred.) Testosterone propionate (oreton* in sesame oil) was given intraperitoneally (0.5 mg.) to two pregnant mice and two male mice and subcutaneously (0.03 mg.) to two litters of newborn mice, of which four were studied 24 to 48 hours after receiving the drug.

Results

The results can best be presented in related groups and then discussed in the subsequent section. The broader inferences of the results of these experiments coupled with some drawn from previous experiments [2] are integrated in Figure 4 and the accompanying Table.

Anoxia Experiments in Newborn Animals

(*a*) *Simple Anoxia.*—When newborn mice and rats were subjected to asphyxia in nitrogen they almost always survived 35 to 50 minutes. Some were clearly dead after these intervals and could not be revived; others revived spontaneously when put back into air, even after 50 minutes. In all the experiments, including those with fed and starved animals, only four animals died in less than 30 minutes. These were just born and may not have become fully adjusted to the extrauterine environment, for they were not very active at the time asphyxia was started. One survived 15 minutes and the others 25 minutes.

When immersed in nitrogen the newborn animal continued to breathe regularly for a few moments, then gasped and struggled irregularly for a minute or two, urinated, and turned cyanotic. From the onset of cyanosis it remained motionless except for a gasp every few minutes. If jostled it gasped and moved its limbs briefly. If removed during the asphyxial period and stimulated it would breathe quickly, become pink, and resume normal activity. The heart continued to beat during the asphyxial period [10] and might continue to beat for several minutes after the animal could no longer be revived by stimulation or by artificial respiration. The heart rate during asphyxia was slower than normal; it could be visualized moderately well through the animal's chest wall. Histologic studies of the animals which were revived after 30 to 50 minutes of asphyxia, returned to the mother, and killed 24 hours later showed no lesions in any organs except in one or two instances, in which there were rare necrotic cortical or brain-stem neurons and neuroblasts

10. The erroneous statement appeared in the literature that these animals cease all circulatory and respiratory activity during asphyxia. Not only could the slowly beating heart be observed in the intact animal, but in a number of additional animals (not counted here) the chests were incised at various intervals to confirm this finding.

at the medial angles of the lateral ventricles (as noted under "Materials and Methods"). In brief, prolonged asphyxia caused virtually no damage or rarely damaged cortical neurons and possibly some neuroblasts and maturing oligodendroglia cells at the inner angles of the ventricles at the junction of the corpus callosum. Glycogen stains (McManus' periodic acid-Schiff reagent method) showed that during the asphyxial period glycogen disappeared, or was partly depleted, from the heart, and a considerable amount disappeared from the liver. Material giving a positive glycogen stain in cartilage, skin appendages, choroid plexus, and skeletal muscle did not change appreciably. The material staining positively for glycogen in brain-stem neurons and showing faintly in the brain ground substance was absent or reduced after asphyxia, but more studies are needed to verify that this change was greater than the normal variability inherent in the stain.

Animals 10 days old survived only a few minutes, and those 16 days old were essentially like adults, surviving about one and one-half to three minutes.

(*b*) *Anoxia in Combination with Other Agents.*—In brief, iodoacetate halted anaerobic survival abruptly, and fluoride or hypoglycemia did so a little less effectively. All the other agents failed to do so consistently, and it can be said that iodoacetate, insulin, and fluoride behaved quantitatively much differently from the other agents.

When an animal was treated with a sublethal or slowly lethal dose of iodoacetate 13 minutes to 2 hours before asphyxia, it died quickly when immersed in nitrogen. Death occurred in 3 minutes when the iodoacetate was given only 13 and 17 minutes before immersion in nitrogen. If the iodoacetate was allowed to act for one-half to two hours before asphyxia, death resulted in about two minutes. The animal gasped, struggled, and turned cyanotic within a minute or so, and the heart slowed and a few moments later stopped beating. A few skeletal-muscle twitches occurred, sometimes a few seconds after the heart had stopped. Survival then was essentially the same as that of adults. Control animals given the same doses of iodoacetate were unaffected in air and when killed 24 hours later showed no pathologic changes. When animals were given small enough doses of iodoacetate (less than 10 mg. per kilogram) to permit survival in nitrogen and examined 24 hours later, they had sustained necrosis of scattered brain-stem neurons (but not neuroblasts) similar to that seen with certain other agents, especially insulin.

Chlormercuribenzoate and oxophenarsine hydrochloride failed to interfere with survival in nitrogen. Cortisone appeared to shorten survival of three animals to 15 to 20 minutes, but the remainder survived more than 30 minutes and usually 40 to 55 minutes. In several cortisone-treated animals the heart kept beating slowly for 15 to 20 minutes after opening of the chest following an asphyxial period of about 30 minutes. When stained for glycogen these hearts showed that it had disappeared. Thus it appears that cortisone did not usually affect anaerobic survival, and the short survivals could have been due to chance.

Pathologic examinations also failed to disclose consistent evidence that the sulfhydryl reagents were interrupting anaerobic glycolysis. The lesions produced by anoxia plus chlormercuribenzoate or oxophenarsine hydrochloride were usually not different from those caused by these chemicals alone, but in one animal the number of necrotic brain-stem and cortical neurons was in excess of that seen after oxophenarsine or asphyxia alone. Cortisone and corticotropin did not produce recognizably

more damage when combined with anoxia than they did alone. However, it might be difficult to distinguish pathologically between degrees of effects of cortisone and cortisone plus asphyxia, because there is enough individual variation to cover up slight but significant differences if real.

Fluoride appreciably lessened survival in nitrogen. Death occurred in about 15 minutes except in one animal, which lived 25 minutes, when the chemical effect was allowed to develop for more than half an hour and combined with anoxia. Fluoride in vitro acts principally on a step in anaerobic glycolysis near that inhibited by iodoacetate—it inhibits enolase, which catalyses the formation of phosphopyruvate.

Insulin also shortened survival. Of the 14 newborn animals which received insulin and were asphyxiated, 11 died in about 15 minutes, 1 in 20, and 1 in 30, and 1, revived after about 35 minutes, survived.

Fluoroacetate did not shorten survival in nitrogen, nor did 150 r given as described. However, in several fluoroacetate-asphyxia animals neuron necrosis was judged to be greater than that seen after single doses of fluoroacetate alone but comparable to that following multiple doses. Anoxia did not prevent x-rays from damaging neuroblasts, but it did lessen the usual destructive effect. (Anoxia has been shown to protect against radiation effects in certain other circumstances.) Brain-stem neurons showed no damage, so phosphoglyceraldehyde dehydrogenase was not appreciably affected. Possibly this failure was due to the lessened effect of radiation in the absence of oxygen, or perhaps the sulfhydryl group of the enzyme is simply not vulnerable to any of the agents that seem to simulate radiation.

(*c*) *Glycogen Studies.*—As already noted, glycogen left the heart and liver in newborn animals during asphyxia, a fact suggesting that it was the source or one of the sources of anaerobic energy for survival in nitrogen. Animals that survived anoxia with concurrent doses of cortisone also showed depletion of cardiac glycogen and variable decreases of liver glycogen.

Insulin with asphyxia (fatal in 15 or 20 minutes) in two animals examined with glycogen stains was followed by only scant or partial depletion of glycogen in the heart and liver.

HYPOGLYCEMIA EXPERIMENTS IN NEWBORN ANIMALS

Insulin given to newborn animals caused their blood sugar to drop substantially, though not as low in those tested as in adults. Two normal newborn (2-day-old) rats and one mouse showed a blood sugar level of 107 to 115 mg. per 100 cc.; insulin caused this level to drop to 41 to 68 mg. in four newborn rats in four hours. Two of these animals had been starved 24 hours.

The newborn animals did not show symptoms during hypoglycemia, yet they did show moderate to severe necrosis of brain-stem neurons 24 hours later except in one instance, and a few cortical neurons and scattered spinal neurons, especially in the anterior horns, were sometimes necrotic (Fig. 1). Most of the two litters starved from birth, then given insulin, and not returned to the mother died, as noted, in 16 to 24 hours. Three examined in the moribund state showed the same pathologic picture as the others.

It is pertinent to note here that hypoglycemia in pregnant animals, as previously reported [2] and as confirmed in more recent experiments, did not selectively damage the fetus between the 9th and 20th days, but one litter showed a selective necrosis of cortical and brain-stem neurons with complete sparing of neuroblasts. Hypogly-

cemia never selectively damaged neuroblasts in fetuses and in newborn animals failed to do so except once in slight degree. In this animal a few of the cells at the angles of the ventricles—neuroblasts and maturing oligodendroglia cells—were necrotic, as was rarely the case in asphyxia. This animal had severe necrosis of the brain-stem neurons, and scattered cortical neurons were necrotic. There was nothing of the usual neuroblast picture that occurred after more specific agents. In two of several pregnant animals with severe, prolonged hypoglycemia requiring several revivals with glucose, the fetuses died but showed no selective damage. The mothers

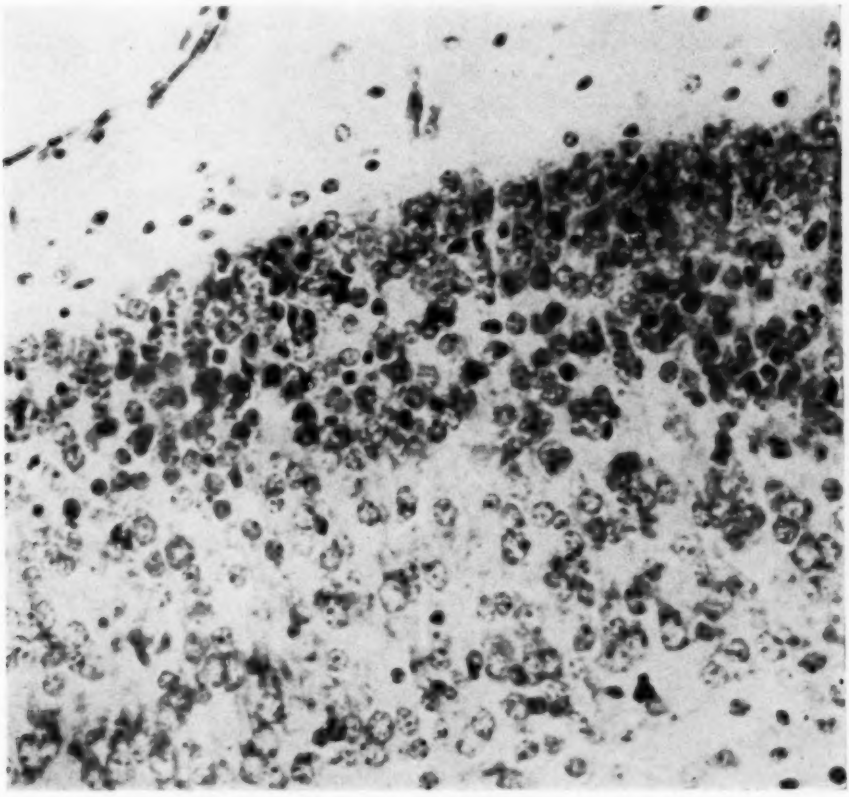


Fig. 1.—Acute cortisone effect on cerebral cortex of infant mouse. All black spots in cellular zone are necrotic neuroblasts. (Hematoxylin and eosin; × 400.)

of the fetuses all sustained cerebral damage, as is the rule after a bout of severe hypoglycemia,[2] and the damage always was sufficient to cause semistupor and intermittent convulsions. It may be assumed that glucose in the fetal blood must also have fallen to low levels, for the substance is freely diffusible across the placenta. Although the fetus must ultimately need oxygen and glucose directly or indirectly for its energy, this stage (death of fetuses) was rarely attained experimentally. The limiting factor of course was usually the greater vulnerability of the mother than of the fetus to hypoglycemia.

CORTISONE AND CORTICOTROPIN EXPERIMENTS

(*a*) *Embryo and Newborn Animals.*—Given to newborn animals, cortisone fairly consistently produced several notable effects. In the nervous system neuroblasts in the outer cortical zones were necrotized in degrees ranging from relatively few to large numbers (Fig. 2) in all but one or two animals studied. To a lesser extent and sometimes not at all, periventricular neuroblasts were damaged, the tendency being for the embryonal cells at the inner angles of the lateral ventricles

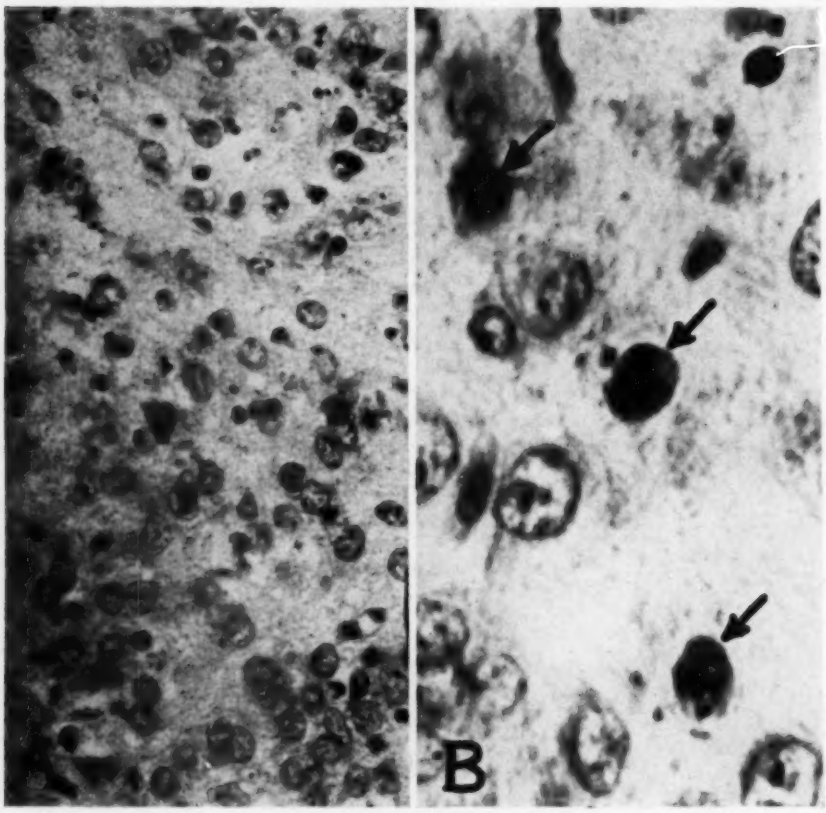


Fig. 2.—Acute hypoglycemic effect on brain stem of infant mouse. *A*, numerous necrotic neurons in various degrees of disintegration are evident as black spots, granules, and smudges. (Hematoxylin and eosin; × 500.) *B*, three necrotic neurons with both nuclear and cytoplasmic remnants are indicated by arrows, and nuclear remnant of a fourth is in focus above. (Hematoxylin and eosin; × 2,000.)

to be vulnerable, as in mild radiation injury. These neural effects were well developed in less than 24 hours. When cortisone was given to mice in the latter part of pregnancy the same pattern sometimes developed, but not always. Gestations of one to nine days in animals to which cortisone was given came to normal completion, though sometimes slightly prematurely, as noted. Corticotropin produced a

similar picture in the newborn and in some instances caused severe necrosis of neuroblasts and young cortical and brain-stem neurons in the fetuses. Repeated doses of cortisone to pregnant animals during the last trimester brought about great debility or death of the fetuses in one litter, accompanied by widespread damage to the nervous system, and a less severe pattern in others. Repeated doses of corticotropin to one pregnant animal resulted in damage not only to the nervous system of its fetuses but to certain mesenchymal elements as well. No damage was seen in early embryos, and mothers treated with cortisone on any day of gestation up to the 14th were delivered of what seemed to be entirely normal young. Although this report deals essentially with acute effects, a gross change in newborn mice (and rats) began so soon after cortisone that it may be mentioned briefly. Within two days it was evident that the animals' hair failed to grow, the skin became loose, the head and ears did not grow as rapidly as those of litter mates, and in the next day or two the face and head were obviously shorter. These animals did not crawl as easily as normal ones and were generally more squat in appearance. Some had convulsive episodes in the second or third week. Resumption of growth after one to three weeks or death was the outcome. (A detailed study is in progress to compare gross cleared skeletal mounts of these animals with the pathologic and histochemical changes in bone at daily intervals up to young adult life and to follow the abnormal growth of the nervous system and the skin. Preliminary studies show that the brain changes result not only from acute destruction of building blocks—neuroblasts—but also from a more subtle disturbance of growth. The latter is probably attributable to the persistence of slowly absorbed cortisone. These studies will be compared with related effects of radiation and other hormones, and rats and mice will be compared.)

(*b*) *Adult Animals.*—Cortisone, corticotropin, testosterone, and diethylstilbestrol caused pathologic changes in the adult brain. These changes consisted of occasional neuron necrosis in the striatum or cerebral cortex (Fig. 3); hyperchromicity of nuclei of oligodendroglia, microglia, and endothelial cells, and sometimes staining of the cytoplasm of many neurons throughout the basal nuclei and interbrain heavier than that usually seen in control material. The hyperchromicity of such cells as the microglial cells, oligocytes, and endothelial cells (as noted under "Materials and Methods") might be dismissed as nonspecific and no different from that which follows the use of many drugs and reagents given in doses insufficient to cause irreversible damage. However, after use of these hormone and hormone-like substances there was also occasional disintegration of nuclei of capillary and large vascular endothelial cells, rarely microglial, and sometimes actual necrosis of oligodendroglia. The changes were not unlike some of those seen in acute form after heavy doses of ionizing radiation to the nervous system.[2] In addition, occasionally there was found a microglial cell in mitosis, an occurrence not seen in the normal animal.

Although the hormone dose given was massive, it is likely that only just so much is assimilated and that the effect was not appreciably enhanced by the size of the dose. No significant difference between 0.25 mg. and 12 mg. could be detected when that range was employed. Thus there is a limiting factor, probably of absorption and solution, of these substances, and the dose, above a certain range, did not

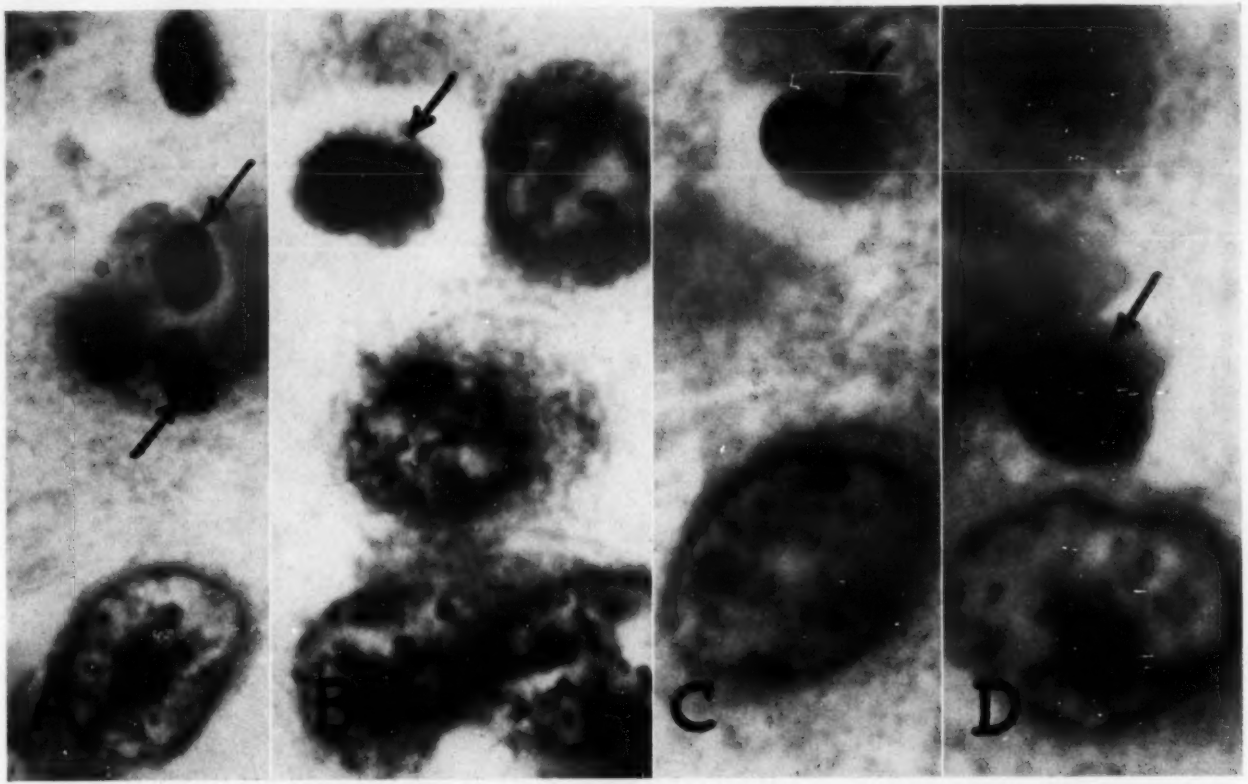


Fig. 3.—Acute cortisone effect on cerebral cortex of adult mouse. *A*, necrosis of two satellite oligodendroglia cells. (Hematoxylin and eosin; × 3,500.) *B*, necrosis of neuron in Area 51 of cortex. (Hematoxylin and eosin; × 3,500.) *C*, necrosis of oligodendroglia cell. (Hematoxylin and eosin; × 5,000.) *D*, necrosis of neuron in Area 17 of cortex. (Hematoxylin and eosin; × 5,000.)

increase the effect. In contrast, the effect of radiation is more proportional to the dose, and relatively high doses are needed to damage the adult nervous system as compared with effects on the embryo.

Lymphoid tissue was little or not at all affected in these experiments, even after heavy doses of cortisone, and the deep crypt cells of the gut, which are so easily damaged by radiation, rarely showed damage.

No differences between males and females or between rats and mice were noted. There was no special distribution of lesions. Estrone produced no visible changes.

To exclude as far as possible effects that might be attributed to water retention, eight adult male and female mice were given massive doses of cortisone (12 mg.) subcutaneously and allowed food and water ad libitum. Their weight, recorded twice daily for three days, did not change measurably from what it was before treatment. Weights were measured to 0.1 gm.

SULFHYDRYL-REAGENT AND OTHER STEROID AND INHIBITOR EXPERIMENTS

The combination of oxophenarsine hydrochloride, chlormercuribenzoate, iodosobenzoate, porphyrindin, and iodoacetate, each in a dose of one-fifth the sublethal amount for adult animals, resulted in one litter in widespread necrosis of neuroblasts and retinal neuroblasts. Six of the eight fetuses were dead in this litter, a fact indicating that the "sublethal" dose had been attained. More mature neurons and lymphoid tissue were damaged rarely. As in similar experiments, not all fetuses were affected to the same degree, some being moderately affected and others mildly so, while some were dead. In two litters no appreciable damage occurred, and none of the fetuses was dead. This combination of chemicals minus the porphyrindin was also given, and some necrosis of neuroblasts resulted.

The combination of oxophenarsine hydrochloride and iodoacetate given to five pregnant mice failed to produce any selective damage. Death or moribund sluggishness occurred in two litters, indicating that the combination had been pushed to the limit. Thus iodoacetate again failed to act like the other sulfhydryl reagents.

The single animal given porphyrindin revealed some necrosis of neuroblasts in its young.

Chlormercuribenzoate or oxophenarsine hydrochloride was given to newborn mice and rats—in single or repeated doses—and there resulted varying degrees of necrosis of neuroblasts, chiefly of the outer cerebral cortical zones, with little or none of the deep masses. The pattern was similar to that due to cortisone and corticotropin except that brain-stem neurons were not affected.

Fluoroacetate caused pathologic lesions similar to those by the sulfhydryl reagents by necrotizing the cortical and some periventricular neuroblasts, but it also tended slightly to damage the more mature cells. To examine this potentiality further, repeated doses were given. These over a period of three hours caused severe cortical-neuroblast damage, and necrosis of a good many cortical and brain-stem neurons. The trend of these fluoroacetate experiments indicates that a prolonged duration of action favors damage to both neonatal neurons and neuroblasts (see "Comment" hereafter).

Some data on the effects of diethylstilbestrol and testosterone on immature animals have been obtained and can be presented because they possibly relate to

cortisone, sulfhydryl-reagent, and radiation effects. Both agents, when given to pregnant and newborn animals, caused some damage to neuroblasts. The damage in experiments thus far has been slight and inconstant within a given litter. Damage to the brain-stem neonatal neurons in some testosterone-treated term fetuses and newborn animals was fairly severe, but in others it was slight.

OBSERVATIONS ON PLACENTAS

There was no pathologic evidence that injury to the placenta caused fetal damage. Comparison of effects on newborn animals with those on fetuses excluded placental-barrier effects for practical purposes in these experiments. The possible barrier to steroids in early gestation is under study.

COMMENT

In these experiments various forms of metabolic inhibition were administered to living animals up to a point that failed to kill the animal but selectively killed part of it. The part damaged was therefore the part that was most vulnerable to the agent, whatever the mechanism. This much is direct experimental observation. The reasons why certain parts are selectively affected is a complex problem that has been outlined in the introduction to this report and in previous publications. By using different agents that in vitro inhibit certain enzymes or enzyme systems and finding that they produce a similar effect, one can surmise that their action on the animal is primarily an enzymatic inhibition. One example already given is the damage to white matter by different agents that inhibit cytochrome oxidase, which seems to constitute an enzymatic bottleneck for white matter, either by action on the fiber or, as seems increasingly likely, also on the oligodendroglia.[2] Another is the deleterious effect on neuroblasts of agents that affect sulfhydryl groups or interfere with nucleic acid metabolism, as shown in current and previous experiments.

The method of these experiments compares different cells with each other and the same cell with itself at different stages of development. It does not so much tell about the absolute mechanisms of cell metabolism as indicate that differences exist. It is these differences that make a muscle cell different from a lymphocyte and a neuron unlike a neuroblast. It is these differences that determine the variety of disease processes that affect the body and the different response of normal and neoplastic tissues to chemical agents and interruptions of various phases of metabolism.

During development the neuron passes through several stages of maturation. It begins as primitive germinal epithelium which may be called neurectoderm. From this neuroblasts develop. These are rapidly differentiating cells that may or may not divide—their mitotic activity is scarcely comparable to that of their germinal precursors. They are growing in a differential way rather than in a divisional way. Their ultimate differentiation is into the adult cells of the nervous parenchyma.[11] Concern here has been largely with the line of differentiation leading to the neuron.

11. In the earliest stages it cannot be predicted what will be the ultimate differentiation of these neurectodermal and neuroblastic cells. Morphologically, by study of successive embryologic stages one can predict, for example, that the majority of neuroblasts in a given area will become neurons and a few oligodendroglial cells. Until they show their colors they are like all undifferentiated cells, only statistically predictable.

These studies indicate that the development can be divided approximately into four stages, each having its own characteristics, based on metabolic (and of course morphologic) differences. They are neurectoderm, neuroblast, neonatal neuron, and adult neuron. Some differences in response between the morphologically more primitive neuroblasts in the deep periventricular basal masses and those in the outer developing cortex have been noted. Also, the cells in the corpus callosum at the angle of the ventricles in the newborn that are in part developing oligodendroglia may behave a little differently. For the present the four stages are useful.

Neuroblasts are present from about the ninth day of gestation in fetal mice and rats to nearly three weeks after birth; germinal epithelium continues active well toward term, and some is present for a period in neonatal life. Recognizable neurons develop early in fetal life in the brain stem, toward term in the deeper layers of the cortex and striatum, and progressively in all parts after birth. Some neuroblasts persist in the subependymal regions of the lateral ventricles till young adult life. Thus the developing nervous system offers an unusual spread of its development for experimental purposes.

Developmental Metabolism of the Neuron *

Agent	Neurectoderm	Neuroblast	Neonatal Neuron	Adult Neuron
Anoxia and cytochrome oxidase inhibitors	—	—	—	+
Hypoglycemia	—	—	+	+
Anoxia plus iodoacetate or fluoride	—	—	+	
Steroid	—	+	+	(+)
Radiation and radiomimetics	—	+	—	—
SH reagents	—	+	—	—

* Plus (+) indicates that the agent damages the cell; minus (—) indicates no damage; plus in parentheses (+) indicates minimal effect (see text and Figure 3).

Based on differences, some of the principal characteristics of these stages may be outlined and then empirically summarized as in the accompanying Table. Neurectoderm was unaffected in any of the experiments by the agents used (except by unreasonably high doses of radiation, nitrogen mustard, and triethylene melamine). Therefore its metabolism can be characterized only in a negative way. Neuroblasts were extremely sensitive to ionizing radiations of various sorts,[2] mustard, triethylene melamine, and "aminopterin." Cortisone and sulfhydryl reagents were often less effective but in a number of instances produced severe widespread necrosis of neuroblasts. In the newborn the tendency of these agents was to damage the cortical neuroblasts and spare to a greater or less extent the deep masses of earlier-stage cells. Other steroids produced the same pattern but less effectively (in preliminary experiments). Both neurectoderm and neuroblasts markedly resisted anoxia and hypoglycemia, as compared with effects in adult cells.

The newborn rat and mouse displayed some interesting metabolic characteristics in whole and in part. As a whole they were remarkably resistent to anoxia, surviving three-fourths of an hour or more in nitrogen without oxygen and suffering virtually no permanent damage. This survival in oxygen could be abruptly cut off by iodoacetate (and less abruptly by fluoride), as previously noted by Himwich, who deduced from these and other considerations that the newborn could use glucose aerobically or anaerobically. This optional use of two pathways was demonstrated

further by the fact that with iodoacetate or fluoride the animal could live in air. Himwich's work was carried further by the experiments reported here. Animals given a sublethal combination of anoxia and iodoacetate showed necrosis of brain-stem [12] neurons, demonstrating that they depended on anaerobic glycolysis. This observation was further supported by the facts that hypoglycemia damaged these neurons in the newborn, iodoacetate and fluoride alone did not, and during asphyxia glycogen was used up from the heart and liver.

Death of the neurons and death of the total animal in these circumstances are probably due to different degrees of the same interruptions of glucose utilization. Iodoacetate plus anoxia profoundly affects the brain, as demonstrated by Himwich. He showed that newborn rats when decapitated under normal circumstances exhibited prolonged gasping motions. They gasped only a few moments when they were given iodoacetate in advance and then decapitated, which produced anoxia. Experiments here, however, indicate that the heart also is an important factor, for it too stopped with iodoacetate plus asphyxia. Survival in nitrogen is, then, a composite process.

Neonatal neurons were radioresistant, but cortisone, other steroids, and, to a minor extent, some sulfhydryl reagents damaged them. The cortisone effect, examined frequently, was sometimes severe. Thus the dual action of steroids in acutely damaging both neonatal neurons and neuroblasts poses the problem of dual biochemical effects. Clinical, physiologic, and biochemical studies indicate that cortisone (and some other steroids) may act at more than one site, suppressing glucose metabolism high in the metabolic path (possibly at the hexokinase [13] level), and a system of sulfhydryl enzymes concerned with growth.[14] In vitro studies have, in fact, implicated a number of enzymes. It might be postulated that cortisone regulates, by suppression, an integrated chain of sulfhydryl enzymes that are concerned with the breakdown of glucose to yield energy for synthetic processes on the one hand (neuroblast) and functional processes on the other (neuron), and therefore both cell types could be affected. Cortisone, under these experimental circumstances, probably acts for some time in contrast to a number of the agents employed, but this inevitable discrepancy of comparing short- and long-acting agents does not explain the fact that deprivation of glucose damaged neurons but failed to damage neuroblasts.

The adult cerebral neuron, as is well known, is susceptible to processes which interrupt aerobic breakdown of glucose. Such neurons utilize almost entirely some other path than the Embden-Meyerhof scheme. (Differences amongst nerve cells at various levels and differences between cerebral white and gray matter are, on present evidence, subdivisions of this general observation, that adult brain depends on aerobic utilization of glucose as its principal substrate.) The fact that cortisone and other agents affected the adult brain neurons might at first be explained by the concept of suppression of glucose catabolism,[8] but certain similarities to radiation effects (interstitial and vascular cell changes) again support the dual or multiple mechanism concept. A common meeting ground for these seemingly divergent effects of steroids (cortisone) might lie in suppression of enzymes concerned with the utilization of energy-rich phosphate compounds. Hexokinase governs a phosphorylating step in

12. The most active region of these newborn brains is the brain stem.[6]
13. Possibly a sulfhydryl enzyme.
14. Anderson and others.[7] Gordan, Bentinck, and Eisenberg.[8]

glucose break-down, and biochemical evidence [15] suggests that acute radiation effect may be related to inability to couple energy-rich phosphate compounds (adenosine triphosphate) to synthetic functions. To the neuroblast the latter could be important; to the neonatal neuron glucose phosphorylation is very important. The adult cerebral neuron, by using pathways of aerobic glycolysis, would possibly be less dependent on phosphorylations.

It is appropriate at this point to consider the source and mechanism of utilization of the energy for anaerobic metabolism in the fetal and the newborn, one of the real problems of this study. In one respect the energy sources seem to be different, glucose for the neonatal neuron and something else for the neuroblast. Possibly adenosine triphosphate and phosphocreatine are built up in quantity during aerobiosis to be used continuously, or in the absence of oxygen they allow life to coast through the anoxic phase, whatever the substrate. The process has a time limit of a little less than an hour, however, for the neonatal neuron and an unknown longer period for the neuroblast. Undoubtedly the "borrowed anaerobic time" available to the newborn as a whole is augmented by other factors. One is the inherently lower metabolic rate of the brain of the newborn as compared with the adult, and another may be the extreme economy of movement that accompanies the asphyxial phase. The small amount of glycogen in the brain is probably not important, since glucose and other glycogen stores are available for glycolysis and are used. Although the newborn has a low metabolic rate, it can still get only about one-tenth of the energy from anaerobic glycolysis that it can get from the complete aerobic oxidation of the same substrate. Some explanation other than the Embden-Meyerhof scheme of anaerobic glycolysis seems necessary.

The actions of fluoroacetate may possibly furnish a clue to some of the mechanisms of anaerobic energy. This chemical damaged both young and old neural elements, and asphyxia increased its effect in the newborn. Phosphorylations are essential to the breakdown of glucose anaerobically, and energy-rich phosphate compounds (adenosine triphosphate, phosphocreatine?) come to mind again as the only likely storehouses [16] that might be utilizable during anaerobic survival for both the neuroblast and the glucose-using neonatal neuron. Increasing evidence suggests that energy from the Krebs cycle yields this energy to such compounds during aerobiosis. Fluoroacetate interrupts this process, in part at least, by being transformed into a fluorotricarboxylic acid. This compound, according to Sir Rudolph Peters' latest investigations,[17] results in sidetracking cis-aconitate into citrate, which accumulates. Fluoroacetate also blocks acetate indirectly, although the Krebs cycle and condensation of acetate and oxalacetate are not wholly stopped. This may be why neuroblasts, neonatal neurons, and to some extent adult neurons may be affected by this compound, namely by blocking of a process on the one hand essential to immediate phosphorylation of glucose and on the other a delayed source of anaerobic energy. The formation of energy-rich phosphate bonds and release of the energy to synthetic

15. Ashwell, G., and Hickman, J.: Effects of X-Irradiation upon Enzyme Systems of Mouse Spleen, Proc. Soc. Exper. Biol. & Med. **80**:407, 1952.

16. Flexner, J. B., and Flexner, L. B.: Biochemical and Physiological Differentiation During Morphogenesis: VII. Adenylpyrophosphatase and Acid Phosphatase Activities in the Developing Cerebral Cortex and Liver of the Fetal Guinea Pig, J. Cell. & Comp. Physiol. **31**:311, 1948.

17. Peters, R. A.: The Puzzle for Therapy in Fluoracetate Poisoning, Brit. M. J. **2**:1165, 1952.

and glycolytic processes may become an important focus in explaining some of the effects that have been seen in these animals, after use of this drug and cortisone.

Finally, as a prelude to future studies, these findings can be put down in speculative fashion in model form (Fig. 4). In schematizing the usual energy metabolism diagrams into a simplified form, two pathways of glucose breakdown leading down to the Krebs cycle and another pathway arbitrarily to the left can be indicated, showing that energy from the cycle as well as some building blocks from two and three carbon components are directed into the channels of nucleic acid and protein synthetic processes. (Exactly how energy is coupled into synthesis is of course basically unknown.) All cells probably possess the same basic pattern, but in adult brain the aerobic glucose metabolism is the chief path, and synthesis relatively small, except so far as function is concerned. The neuroblast is concerned chiefly with synthesis (differential growth) and of course needs energy supplies, but it seems to be able to use a delayed-action form of it when necessary. The neonatal neuron lies somewhat between. In the diagram the heavy pathway will signify rather the outstanding

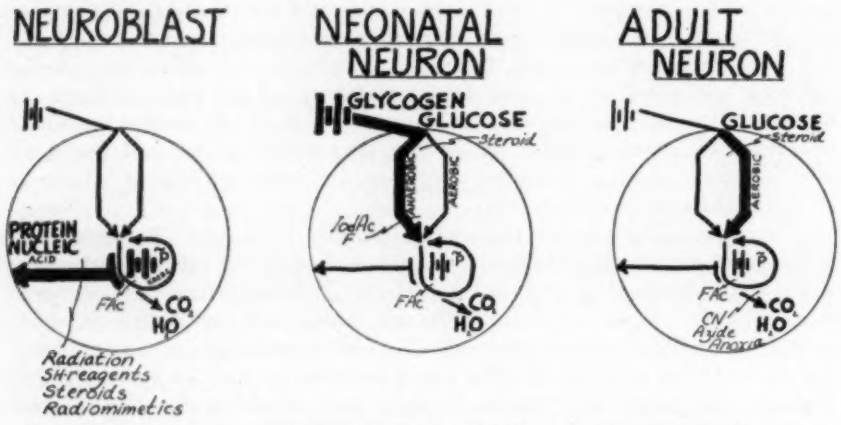


Fig. 4.—Schematic representation of changing metabolic pathways during development of nerve cell. Differences of especial interest, based on experiment, are emphasized by heavy arrows.

characteristic than any relative "size" of metabolic pathways. The storage battery sign is for the anaerobic energy stores. Its position in the Krebs cycle, like the entire model, is tentative; the arrangement, however, admits the possibility that it may be a vulnerable point for neonatal neurons and neuroblasts. Though the steroid action is shown to act possibly at two seemingly divergent points, the possibility is not discarded that it may act on the formation and utilization of energy-rich phosphate compounds which may be built up and broken down by enzymes with sulfhydryl groups.

SUMMARY

Adult, pregnant, and newborn rats and mice were subjected to various forms of metabolic inhibition, and the acute effects were studied pathologically. The results were compared with previously obtained data of a related nature, and a characterization of the metabolism of the nervous system (nerve cell) as it developed from embryo to adult was made on a cellular basis.

The earliest stage, neurectoderm, was invulnerable to a wide variety of metabolic interruptions. The neuroblast was resistant to anoxia and hypoglycemia but was sensitive to destruction by sulfhydryl reagents, radiation, radiomimetic drugs, and the "antisulfhydryl" action of steroids. Its source of anaerobic energy remained a puzzle. The newborn animal could resist anoxia for nearly an hour without damage. At this stage the neonatal neuron had become dependent on glucose and glycogen, which it used aerobically or anaerobically; consequently it was vulnerable to agents that affected glucose metabolism. It was generally unaffected by those agents that injured the neuroblast except steroids and fluoroacetate. The dual effects of these two agents led to the question whether a common site of action on neuroblasts and neonatal neurons might be on the formation and release of phosphate-bond energy.

The adult neuron was dependent on an immediate supply of oxygen and glucose but was insensitive to most agents that injured the neuroblast.

Some cerebral damage resulted from steroids in the adult, characterized not only by occasional neuron necrosis but by interstitial and vascular cell changes. Both steroids and radiation occasionally damaged oligodendroglia, but the effect was much less than that after use of the cytochrome oxidase inhibitors, which produced oligodendroglia necrosis with severe demyelination.

CLASSIFICATION OF GLANDULAR TUMORS OF SALIVARY GLANDS

Study of One-Hundred Forty-Three Cases

WILLIAM H. BAUER, M.D.
AND
JOHN D. BAUER, M.D.
ST. LOUIS

THE PURPOSE of this paper is to present a classification of benign and malignant glandular tumors of the salivary glands based on their prominent morphologic features. The lack of postsurgical follow-up of many of our cases prevents definite conclusions concerning their clinical behavior. This limitation is offset by the fact that "It is impossible to be dogmatic about them (salivary gland tumors) for they disregard every canon of oncology and continually do the most unexpected things" (McFarland).[1] The grouping of these neoplasms according to their outstanding cells and their arrangement is justified, since "There seems to be a certain reaction of frustration among pathologists which leads to relegation of any odd salivary gland tumor to the broad unfenced field of the so-called mixed tumors" (Stewart, Foote, and Becker).[2] Furthermore, the terminology is confused, as is evidenced in recent publications which consider histologically-different tumors of the parotid gland to be identical and use their names interchangeably.

PERTINENT FINDINGS IN NORMAL SALIVARY GLANDS

A better insight into the diversity of the morphogenesis of these tumors will be gained by consideration of certain biologic potencies and histologic features of salivary gland tissue, as follows:

1. We observed that the low cuboidal epithelium of the intercalated ducts and even the columnar epithelium of the striated ducts possesses the potential plasticity to differentiate into serous, mucous, oxyphilic-granular, clear-cell, sebaceous, and squamous types of epithelium. Schaper and Cohen [3] called the intercalated ducts

Supported by a grant from the National Cancer Institute of the National Institutes of Health, United States Public Health Service.

From the Department of Pathology, Schools of Medicine and Dentistry, St. Louis University (Dr. William H. Bauer), and Snodgrass Laboratory, City Hospital, and Department of Pathology, Washington University (Dr. John D. Bauer).

1. McFarland, J.: The Mysterious Mixed Tumors of the Salivary Glands, Surg., Gynec. & Obst. **76**:23, 1943.

2. Stewart, F. W.; Foote, F. W., and Becker, W. F.: Muco-Epidermoid Tumors of Salivary Glands, Ann. Surg. **122**:820, 1945.

3. Schaper, A., and Cohen, C.: Beiträge zur Analyse des thierischen Wachstums: Über zellproliferatorische Wachstumszentren und deren Beziehungen zur Regeneration und Geschwulstbildung, Arch. Entwmech. **19**:348, 1905.

"zones of proliferation." The occurrence of sebaceous glands in the parotid tissue, recently stressed by Hartz,[4] Lee,[5] Meza-Chavez,[6] and Rawson and Horn,[7] must be explained on this ground.

2. Between the epithelium and the inner surface of the basement membrane of the acini and intralobular ducts lie the myoepithelial cells, which by their contraction propel and expel the secretion. They are stellate or elongated cells, with angular or oval nuclei in an oxyphilic cytoplasm. They are found also in the lacrimal, mammary, ceruminous, and sweat glands, where they play a role in the composition of certain tumors.

3. There are often large islands of lymphoid tissue containing isolated glandular elements in the normal large salivary glands, mainly the parotid gland, and there

Classification of 143 Glandular Tumors of the Salivary Glands According to Their Outstanding Morphologic Features

	Total No.	Site		Microscopically Malignant
Polymorphic adenoma ("mixed tumor")	100	Parotid	89	None
		Upper lip	1	
		Cheek mucosa	1	
		Palate	9	
True adenoma, composed of acinous or ductal epithelial cells	3	Parotid	3	None
Oxyphilic granular cell adenoma (oncocytoma)	5	Parotid	5	1
Papillary cystadenoma lymphomatosum	5	Parotid	5	None
Lymphomatoid adenoma	6	Parotid	6	None
Papillary cystadenoma (water-clear cell type)	2	Palate	2	None
	1	Parotid	1	None
Mucoepidermoid tumor	8	Parotid	5	3
		Palate	2	1
		Lower lip	1	
Adenocarcinoma (cylindromatous, papillary, acanthomatous type)	13	Parotid	8	13
		Submaxillary gland	2	
		Palate	3	

are occasionally inclusions of salivary gland tissue (ducts, acini, and individual epithelial cells) in the preauricular lymph nodes of children and adults. These findings, reported by various investigators and confirmed by us, will be relevant in the discussion of the genesis of papillary cystadenoma lymphomatosum (Warthin's tumor) and lymphomatoid adenoma of the parotid.

MATERIAL AND METHODS

The material consisted of 143 surgically removed tumors: 122 neoplasms of the parotid gland, 16 of the palatal salivary glands, 1 of the mucous glands of the cheek, 2 of the lip, and 2 of the submaxillary gland (Table). No tumor of the sublingual gland has been examined. Study of

4. Hartz, P. H.: Development of Sebaceous Glands from Intralobular Ducts of the Parotid Gland, Arch. Path. **41**:651, 1946.

5. Lee, C. M., Jr.: Intraparotid Sebaceous Glands, Ann. Surg. **129**:152, 1949.

6. Meza-Chavez, L.: Sebaceous Glands in Normal and Neoplastic Parotid Glands, Am. J. Path. **25**:627, 1949.

7. Rawson, A. J., and Horn, R. C.: Sebaceous Glands and Sebaceous Gland-Containing Tumors of the Parotid Salivary Gland, Surgery **27**:93, 1950.

this material was combined with microscopic examination of 83 normal salivary glands of elderly persons who came to autopsy. The ages of 57 of these persons ranged from 61 to 92. The parotid glands of four stillborn infants were also examined.

Though salivary gland neoplasms occur chiefly after the age of 30 and increase in frequency with age, our series included malignant epithelial tumors of the parotid gland of girls aged 10, 12, and 15 years and of the palatal gland of a 24-year-old woman.

We placed emphasis on obtaining many sections from various levels in order to avoid the fallacy of basing the diagnosis on one or few sections. Blocks of the specimens were fixed in formalin or alcohol and stained with hematoxylin and eosin, aniline blue, iron hematoxylin and basic fuchsin, Mayer's mucicarmine, and Sudan IV. Mallory's phosphotungstic-acid hematoxylin stain and Masson's trichrome stain with hemalum-erythrosin-safranine were employed for demonstration of the myoepithelial cells. Frozen sections of a papillary cystadenoma lymphomatosum were stained with thionine in tartaric acid (so-called "inclusion stain" of Feyrter).

RESULTS

1. *Pleomorphic Adenoma.*—Willis'[8] suggestion to replace the misnomer "mixed tumor" by the more adequate term pleomorphic adenoma deserves strong support, since this name indicates the epithelial origin and the great variety of the structural pattern. Our study of step sections showed that the epithelial cells of the intralobular ducts proliferated, subsequently lined duct-like and alveolar spaces, formed cords and solid aggregations or occasionally papillary and cystic structures, and displayed metaplasia into stratified squamous epithelium with keratinization (Fig. 1*A*). A similar process has been observed by Bauer and Byrne[9] in experimentally induced parotid gland tumors in mice. The hypothesis as to the epithelial origin of pleomorphic adenoma of the parotid gland has been strengthened by the work of Favata[10] on cultures of mixed tumors. The presence of mucoid and pseudocartilaginous areas, hyaline cartilage, and even bone could not militate against the epithelial genesis. The myxomatous and cartilage-like ground substance was the product of a combination of epithelial secretion with hyaline changes of the stroma and dissolution of chromatin of disintegrated nuclei. It was due to this composition that in sections stained with hematoxylin and eosin the centers of these areas appeared pink with a margin of bluish-stained material. Distorted epithelial cells with hyperchromatic nuclei embedded in this tissue resembled hyaline cartilage.

Close scrutiny revealed that there were other special epithelial elements which participated in the structure of pleomorphic adenoma. These were the myoepithelial cells (Fig. 1*B*), the role of which in tumor formation has been ignored in the past. French pathologists paid attention to these cells in the formation of tumors, since such cells possess the capacity to proliferate either by themselves or in combination with epithelial cells. Special stains such as Van Gieson's and particularly erythrosin-safranine (Masson) make the myoepithelial cells stand out in the hyperplastic epithelium of the ducts of the mammary glands of elderly women (Günther[11]), in

8. Willis, R. A.: Pathology of Tumours, St. Louis, C. V. Mosby Company, 1948.

9. Bauer, W. H., and Byrne, J. J.: Induced Tumors of the Parotid Gland, Cancer Res. **10**:755, 1950.

10. Favata, B. V.: Characteristics of Mixed Tumors of the Parotid Gland Growing in Vitro, Surg., Gynec. & Obst. **86**:659, 1948.

11. Günther, R.: Myoepitheliale Wucherungen in der Brustdrüse, Arch. path. Anat. **300**:449, 1937.

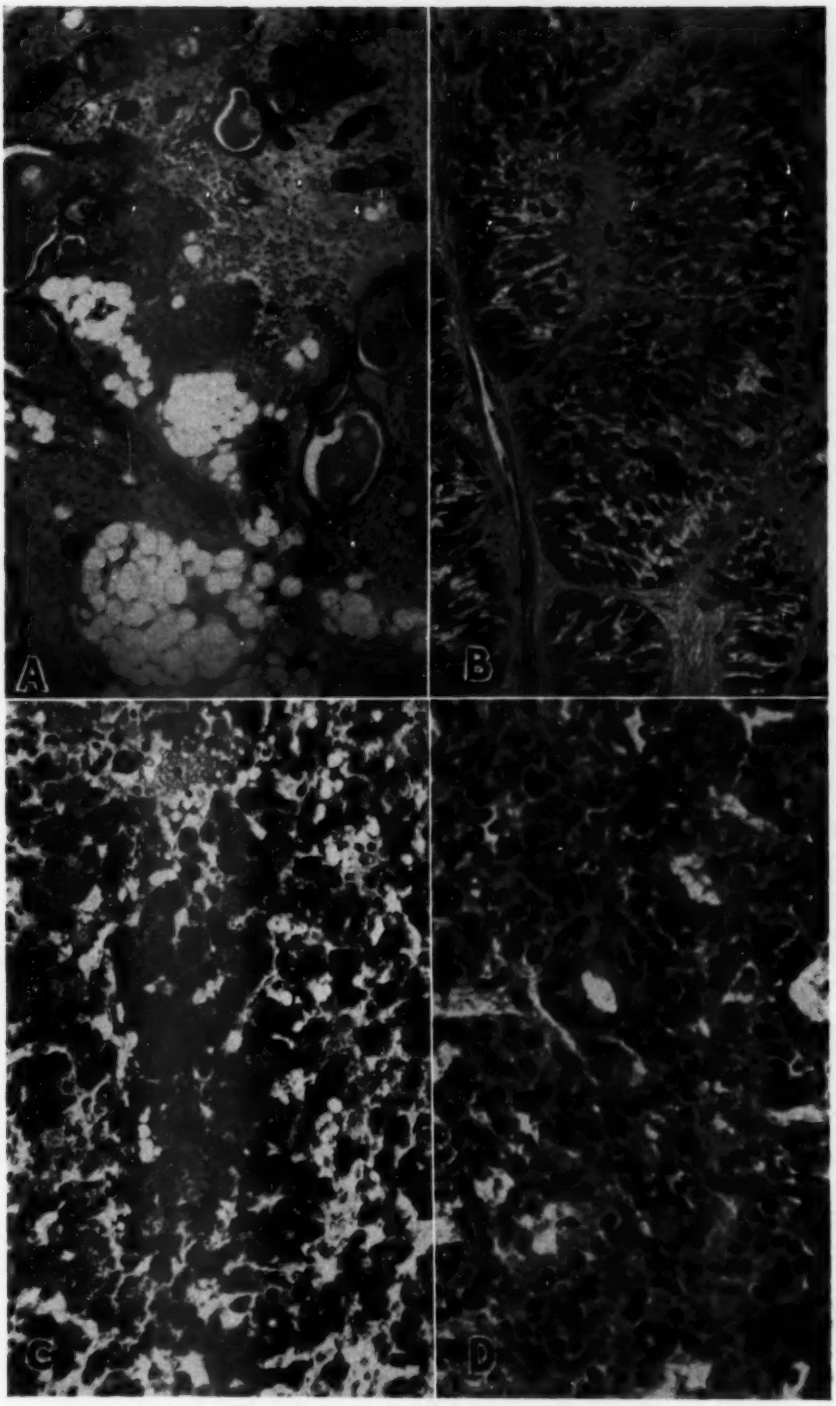


Fig. 1.—*A*, area of pleomorphic adenoma (mixed tumor) of parotid gland showing pseudocartilaginous tissue and acini and ducts in hyalinized connective tissue. × 120. *B*, collections of myoepithelial ceils in "mixed tumor" of palatal salivary glands. × 200. *C*, acinous adenoma of parotid gland. × 140. *D*, canalicular (ductal) adenoma of parotid gland. × 140.

mammary tumors (Hamperl,[12] Kuzma [13]), and in neoplasms of the sweat glands and salivary glands (Sheldon [14]). The usual hyperchromatism of the elongated myoepithelial cells proliferating in the stroma, their polymorphism, and the numerous mitotic divisions may lead to an erroneous diagnosis of malignant tumor (Kuzma [13]). In four pleomorphic adenomas of the parotid gland the myoepithelial cells proliferated into the hyalinized stroma and displayed a wide range of shapes. Triangular cells with processes and elongated cells with dark nuclei in a fibrillar cytoplasm merged with the hyalinized fibrous stroma and stood perpendicular to the lumina of the acini and ducts, maintaining a palisaded arrangement in the areas of dense proliferation. In these areas they very much resembled a neurilemmoma, and yet the aforementioned special stains and the detectable site of their origin proved them to be myoepithelial cells. Sheldon [14] showed their arrangement in interlacing bundles. Of the pleomorphic adenomas studied, only four displayed a definite preponderance of myoepithelial cells but no areas of pseudocartilage. Sheldon [14] called these tumors myoepitheliomas, while Bauer and Fox [15] used the term adenomyoepithelioma because of the adenomatous features.

With regard to the nature of pleomorphic adenoma, we wish to refer to the study of Rawson and associates,[16] who found that almost all of 100 mixed tumors, which they examined and observed, were benign. However, there should not be any doubt that in rare instances a carcinoma may develop in a so-called mixed tumor, as noted by these authors in two additional cases.

2. *Acinous and Canalicular Adenoma.*—In contrast to the common pleomorphic adenoma, true adenomas of the salivary glands, composed solely of unaltered acinous and ductal cells, are rare. Since single sections of a pleomorphic adenoma may display the features of such a true adenoma, step sections are necessary for the diagnosis. The occurrence of this type of tumor has been doubted by some authors, but adenomas of the serous cells of salivary acini and of cuboidal cells of ducts have been described by Lang,[17] McFarland,[18] Škorpil,[19] and others. French pathologists distinguished between acinous adenoma of the parotid and canalicular adenoma of the ductal epithelium. The three cases we observed confirm this differentiation. Two of the tumors were well encapsulated, lobulated growths of the parotid gland which grew slowly over a period of years and were composed of serous cells of fairly uniform shape, densely filled with basophilic granules and

12. Hamperl, H.: Über die Myothelien (Myo-epithelialen Elemente) der Brustdrüse, Arch. path. Anat. **305**:171, 1939.

13. Kuzma, J. F.: Myoepithelial Proliferations in the Human Breast, Am. J. Path. **19**:473, 1943.

14. Sheldon, W. H.: So-Called Mixed Tumors of the Salivary Glands, Arch. Path. **35**:1, 1943.

15. Bauer, W. H., and Fox, R. A.: Adenomyoepithelioma (Cylindroma) of Palatal Mucous Glands, Arch. Path. **39**:96, 1945.

16. Rawson, A. J.; Howard, J. M.; Royster, H. P., and Horn, R. C.: Tumors of the Salivary Glands: A Clinicopathological Study of 160 Cases, Cancer **3**:445, 1950.

17. Lang, F. J.: Pathologische Anatomie der grossen Kopfspeicheldrüsen, in Handbuch der speziellen pathologischen Anatomie und Histologie, edited by Henke, F., and Lubarsch, O., Berlin, Springer-Verlag, 1929, Vol. 5, Pt. 2, p. 1.

18. McFarland, J.: Adenoma of the Salivary Glands: A Report of a Possible Case, Am. J. M. Sc. **174**:362, 1927.

19. Škorpil, F.: Über das Speicheldrüsenadenoma, Arch. path. Anat. **306**:714, 1940.

grouped in acini (Fig. 1*C*). The pattern seemed to imitate the structure of the normal parotid gland, but no ductal elements were found within the tumor. The third tumor showed a dense arrangement of tubules containing eosinophilic material lined by low cuboidal epithelium as it occurs in the intercalated ducts. Only a few terminal serous acini were noted (Fig. 1*D*).

3. *Oxyphilic Granular-Cell Adenoma (Onkocytoma).*—One of the most intriguing neoplasms of the parotid gland is the oxyphilic granular-cell adenoma (onkocytoma). Microscopic examination of four oxyphilic granular-cell adenomas of the parotid glands of persons between 40 and 65 years of age showed the tumors to be composed of lobules of oxyphilic granular cells in solid aggregations, cords, and acini separated by strands of dense fibrous tissue, thin capillaries, and normal parotid tissue (Fig. 2*A*). In many areas the cords branched and encircled small cystic spaces containing either debris of oxyphilic granular cells or hyalinized connective tissue.

The name oxyphilic granular-cell adenoma, suggested by Meza-Chávez,[20] should replace the name "oncocytoma," as it suggests the two paramount features of the tumor: oxyphilic granular cells and an adenomatous arrangement. In spite of the informative work of Hamperl,[21] Ahlbom,[22] Škorpil,[19] Gruenfeld and Jorstad,[23] Tonelli,[24] Meza-Chávez,[20] and others, there has been a promiscuous application of the term "oxyphilic granular cell" and a lack of distinction between oxyphilic granular-cell adenoma and papillary cystadenoma lymphomatosum.

Hamperl,[21] using thionine and cresyl violet stains, described the morphologic features and the occurrence of oxyphilic granular cells in normal and neoplastic salivary glands and in thyroid, parathyroid, pituitary, and adrenal glands. Furthermore, Hamperl,[25] and recently Stout,[26] demonstrated oxyphilic granular cells in bronchial adenomas. Riopelle [27] found in cases of "so-called true kidney hypernephroma" cells with abundant, intensely acidophilic granular cytoplasm reminiscent of "oncocytes." In accord with the findings of these authors, it is our observation that only those large polygonal cells which display a distinctly eosinophilic granular cytoplasm and a well-shaped vesicular nucleus are capable of multiplication by amitotic division. The pyknotic appearance of the nucleus in some of these cells induced Zimmermann [28] to speak of "pyknocytes." Since we found the debris of such

20. Meza-Chávez, L.: Oxyphilic Granular Cell Adenoma of the Parotid Gland (Oncocytoma), Am. J. Path. **25**:523, 1949.

21. Hamperl, H.: Oncocytes and the So-Called Hürthle Cell Tumor, Arch. Path. **49**:563, 1950.

22. Ahlbom, H. E.: Mucous and Salivary Gland Tumors, Acta radiol., Supp. 23, p. 1, 1935.

23. Gruenfeld, G. E., and Jorstad, L. H.: Adenoma of the Parotid Salivary Gland: Oncocyte Tumor, Am. J. Cancer **26**:571, 1936.

24. Tonelli, L.: I tumori di oncociti e gli oncociti nei tumori delle ghiandole salivari, Arch. "de vecchi" anat. path. **11**:413, 1948.

25. Hamperl, H.: Über gutartige Bronchialtumoren (Cylindrome und Carcinoide), Arch. path. Anat. **300**:46, 1937.

26. Stout, A. P.: Cellular Origin of Bronchial Adenoma, Arch. Path. **35**:803, 1943.

27. Riopelle, J. L.: The Nature and Origin of the So-Called True Kidney Hypernephroma, Cancer **4**:789, 1951.

28. Zimmermann, K. W.: Die Speicheldrüsen der Mundhöhle und die Bauchspeicheldrüse, in Handbuch der mikroskopischen Anatomie des Menschen, edited by Möllendorf, W. V., Berlin, Springer-Verlag, 1927, Vol. 5.

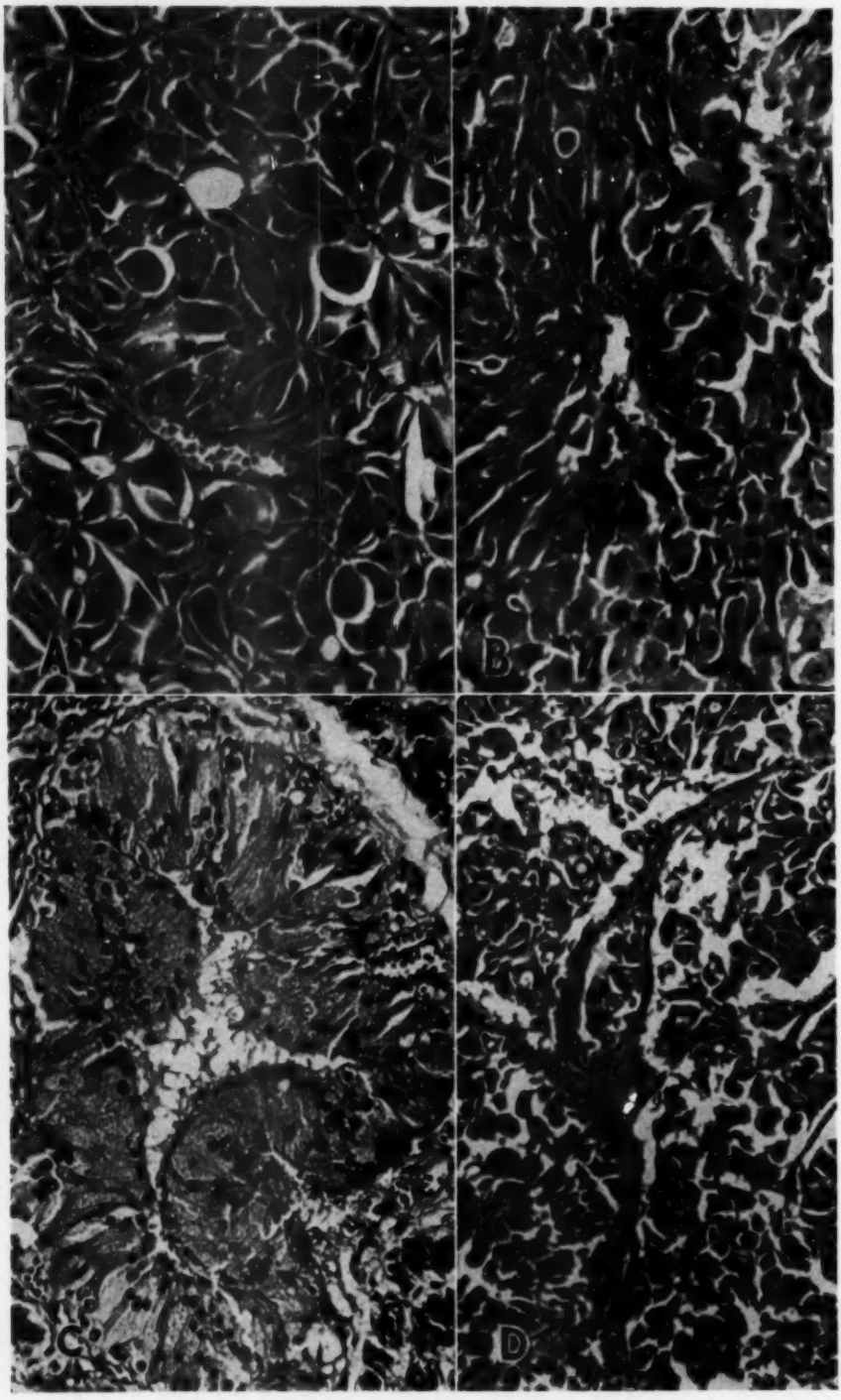


Fig. 2.—*A*, acinous grouping of large oxyphilic granular cells in oxyphilic granular-cell adenoma (oncocytoma) of parotid gland. × 300. *B*, transition of basophilic ductal epithelial cells into large oxyphilic granular cells in oxyphilic granular-cell adenoma of parotid gland. × 250. *C*, oxyphilic granular cells lining duct in normal parotid of 82-year-old-man. × 300. *D*, area of malignant oxyphilic granular-cell tumor of parotid with metastases. × 300.

pyknotic and hyperchromatic cells in the cystic spaces of oxyphilic granular-cell adenoma, in papillary cystadenoma lymphomatosum, and in the dilated ducts of normal salivary glands of old persons, we regard these cells as degenerated forms.

Accepting the outlined criteria for viable oxyphilic granular cells, we were not able to demonstrate such a high percentage of these cells in normal parotid and submaxillary glands of aging and aged persons as Hamperl [21] and others did. We found solid clusters and adenomatous formations or proliferated typical oxyphilic granular cells associated with the ducts in only 8 of 57 salivary glands in an age range of 61 to 92 years (Fig. 2*C*). Our figure comes fairly close to that reported by Meza-Chávez.[20] None of the salivary glands of old people which we examined demonstrated development of oxyphilic granular cells from acinar epithelium.

The origin of oxyphilic granular cells lies in the multipotential cells of the intercalated ducts, as evidenced by the transition of small basophilic cuboidal lining cells into swollen oxyphilic granular cells (Fig. 2*B*). The cause and the mode of the cytoplasmic change are still obscure. We believe that the process is an alteration of the cytoplasm that transforms ductal cells to oxyphilic granular cells capable of multiplication and formation of benign and malignant tumors.

Since no case of a malignant granular-cell oxyphilic adenoma has hitherto been observed—as far as we could ascertain—we report briefly the microscopic description of such a parotid tumor.[29] It had metastasized to the lungs, the liver, the dura, and the pituitary gland. The microscopic features of the metastases were identical with those of the original parotid tumor (Fig. 2*D*). There were large oxyphilic granular cells with two and three well-preserved nuclei, small oxyphilic granular cells, many mitotic figures, necrotic areas, and invasion of blood and lymph channels by neoplastic oxyphilic granular cells. The presence of a few intercalated ducts in the neoplasm indicated replacement of the parotid gland by the tumor.

4. *Papillary Cystadenoma Lymphomatosum (Warthin's Tumor).*—The literature on this tumor of the parotid gland has so increased in recent years that it would be repetitious to discuss in detail the interesting history of this neoplasm, which should be accepted as a clinical and pathological entity. Recently Thompson and Bryant [30] and Stevenson and Hazard [31] covered the subject well and rightly stressed the confusion that still exists concerning the morphology.

The five tumors studied involved the parotid glands of men 48 to 58 years of age. The fact that all five tumors occurred in males is in accord with Thompson and Bryant's [30] report on 23 cases with 22 in males, Hevenor and Clark's [32] discussion of 20 cases with 19 in males, and Stevenson and Hazard's [31] 8 cases with 6 in males.

The parotid gland is the commonest site of involvement. Thompson and Bryant [30] expressed doubt that the submaxillary gland is ever the site of origin of a papillary cystadenoma lymphomatosum by pointing out that the lower pole of the parotid gland

29. Dr. F. Leidler, director of laboratories of the Veterans Administration Hospital, Jefferson Barracks, Mo., provided the clinical and pathological reports.

30. Thompson, A. S., and Bryant, H. C.: Histogenesis of Papillary Cystadenoma Lymphomatosum (Warthin's Tumor) of the Parotid Salivary Gland, Am. J. Path. **26**:807, 1950.

31. Stevenson, G. F., and Hazard, J. B.: Papillary Cystadenoma Lymphomatosum, Cleveland Clin. Quart. **18**:85, 1951.

32. Hevenor, E. P., and Clark, C. E.: Adenolymphoma (Papillary Cystadenoma Lymphomatosum), Surg., Gynec. & Obst. **90**:746, 1950.

33. Footnote deleted.

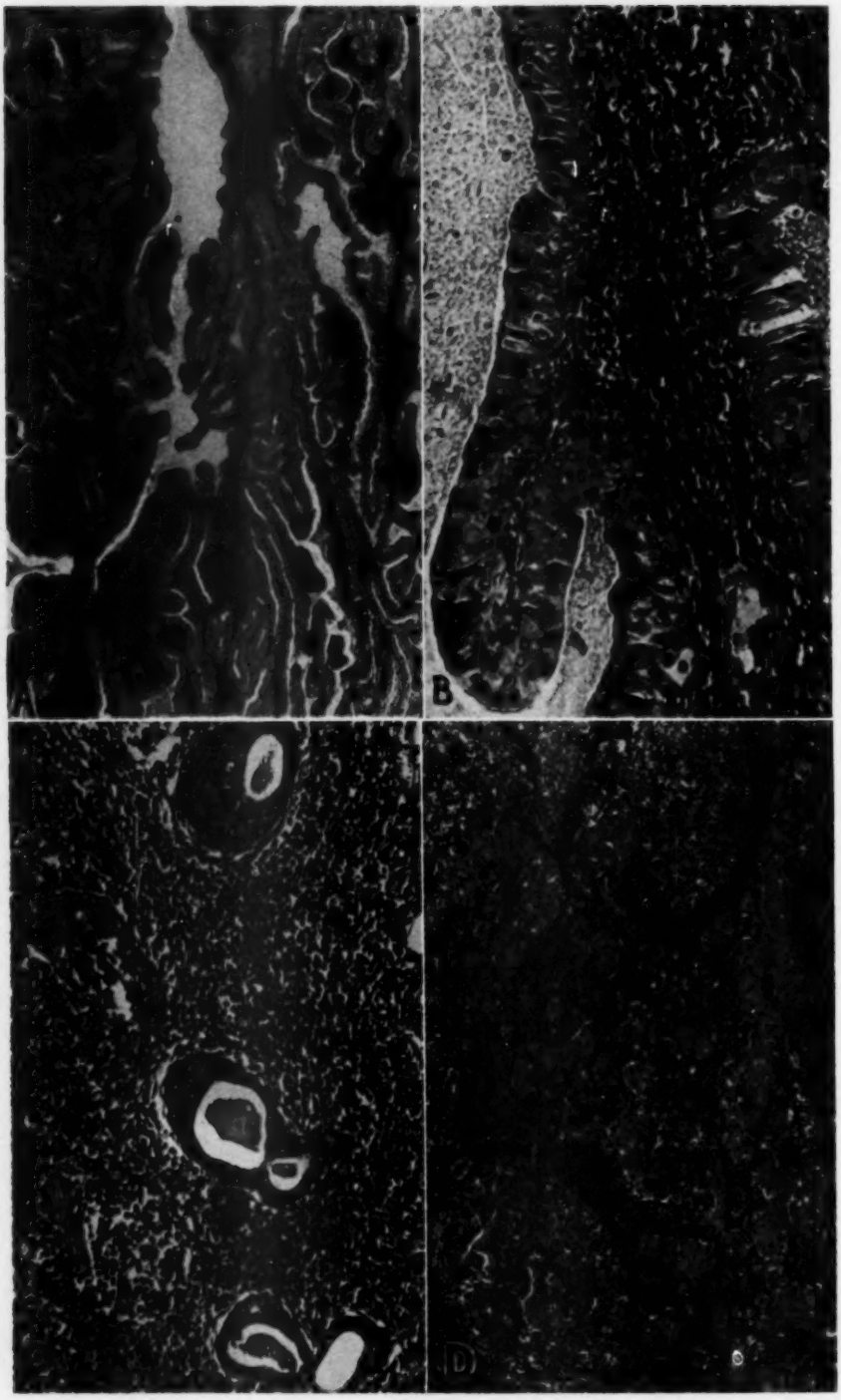


Fig. 3.—*A*, papillary cystadenoma lymphomatosum of parotid gland. × 30. *B*, area of *A* showing cystic spaces lined by high columnar cells with well-preserved nuclei and thread-like cells with pyknotic nuclei. × 200. *C*, lymphomatoid adenoma of parotid gland, showing ducts lined by proliferated epithelium in lymphoid tissue. × 140. *D*, third recurrence of lymphomatoid adenoma of parotid gland. This section shows a remarkable increase of adenomatous structures in lymphoid tissue with germinal centers as compared with the sections of the original tumor.

often meets the submaxillary gland. We observed this situation in microscopic studies of normal parotid glands. However, recently Hevenor and Clark,[32] reported two cases of papillary cystadenoma lymphomatosum in the submaxillary gland and one in the wall of the hypopharynx.

In our cases the growths were surrounded by a delicate capsule of connective tissue containing congested capillaries. Between the capsule and the tumor there was a broad layer of lymphoid tissue traversed by blood-filled spaces. From this lymphoid rim strands of lymphoid tissue with occasional germinal centers branched off and separated interlacing cords and tubules lined by high columnar epithelial cells (Fig. 3*A*). These cords and tubules encircled cystic spaces which contained papillary extensions and eosinophilic material (Fig. 3*B*). The latter was a mixture of epithelial secretion and cytoplasm of disintegrated cells. At irregular intervals individual cells of compressed and filamentous shape with pyknotic nuclei and homogenous very oxyphilic cytoplasm were observed in the row of high columnar epithelial cells (Fig. 3*B*). The so-called inclusion stain of Feyrter [34] revealed a bluish-pink granular cytoplasm in the columnar cells and a reddish homogeneous cytoplasm in the flattened cells. We consider the great majority of the lining cells displaying well-preserved nuclei as the essential elements and the compressed cells with pyknotic nuclei as degenerated.

The cells of papillary cystadenoma lymphomatosum originate from the epithelium of the intercalated ducts. With this fact in mind the question arises—where do salivary ducts enter into a close relationship with lymphoid tissue? Our histologic study of parotid glands and preauricular lymph nodes of infants and adults showed that large accumulations of lymphoid elements are not uncommon in the parotid glands, but parotid ducts with acini are seldom found in adjacent lymph nodes. The finding of parotid elements in lymph nodes could lend support to the opinion of Neisse,[35] Thompson and Bryant,[30] and others that papillary cystadenoma lymphomatosum may develop in these nodes by neoplastic proliferation of ductal epithelium. However, our findings led us to the opinion that papillary cystadenoma lymphomatosum develops within the parotid gland. We believe it unlikely that the few small heterotopic parotid ducts in adjacent lymph nodes give rise to neoplasms of such striking papillary formation.

5. *Lymphomatoid Adenoma.*—We have chosen the term "lymphomatoid adenoma" for six rare neoplasms of the parotid gland in order to distinguish this type of growth from lymphoma and papillary cystadenoma lymphomatosum yet to make clear that it displays adenomatous features in a lymphoma-like tissue.

A search through the literature showed that few such tumors have been observed. They usually have been included in the group of papillary cystadenoma under various names, such as "adenolymphoma," "solid adenolymphoma," "lymphocytic tumor," and "adenoma of preparotid lymph gland." This tumor has also been interpreted as a "lymphoepithelioma," although there is no resemblance to this growth of the nasopharynx.

---

34. Feyrter, F.: Über die chromotrope granuläre Entartung, Wien. Ztschr. Int. Med. **28**:5, 1947.

35. Neisse, R.: Über den Einschluss von Parotisläppchen in Lymphknoten, Anat. Hefte **10**:289, 1898.

Fein [36] called a parotid tumor the structure of which was identical with ours a lymphoepithelioma. Plaut [37] reported three such tumors as salivary lymphadenomas. Lloyd [38] described six such benign neoplasms of the parotid and included them in the group of adenolymphomas, although he admitted striking histologic differences. Kirklin and associates [39] differentiated and called "lymphocytic tumors" those neoplasms of the parotid which were comprised of a "monotonous array of lymphocytes with occasional germinal centers and areas of reticulum cells."

Microscopically the six growths showed normal parotid gland tissue partly compressed and partly replaced by massive collections of small lymphocytes with germinal centers (Fig. 3*C* and *D*). Tubules, acini, and isolated epithelial cells appeared scattered throughout the lymphoid tissue. Many of these tubules and acini appeared multilayered and extremely dilated; others exhibited squamous metaplasia of the lining cells. Collections of reticuloendothelial cells were set in the abundant lymphoid tissue. Fairly large hemorrhages with intracellular hemosiderin in the vicinity were observed in the tissue. Most of the dilated tubules contained red blood cells. The multilayered epithelium of few tubules and acini was infiltrated by lymphocytes, and the lining epithelial cells appeared vacuolated. Large islands of lymphocytes were found within and outside the uninvolved parts of the parotid gland. Broad strands of lymphocytes projected into the normal glandular tissue.

Concerning the origin, we could not decide whether the growth started in the lymphoid islands within the parotid or in one of the parotid lymph nodes. The latter may contain glandular elements. The unknown factor that caused the tumor-like hyperplasia of the lymphoid tissue could account for the adenomatous neoplasia of the included glandular structures.

We regard the "lymphomatoid adenomas" of the parotid gland as a clinical and pathologic entity, the neoplastic nature of which is documented by the recurrences observed and the definite increase of the adenomatous structures in these cases (Fig. 3*D*). The microscopic structure, which differs considerably from that of papillary cystadenoma lymphomatosum, did not exhibit any malignant features.

6. *Papillary Cystadenoma.*—A tumor of distinctive type is the papillary cystadenoma of the major and minor salivary glands described in the French and German literature. In site of involvement, mode of development, and microscopic structure it differs from papillary cystadenoma lymphomatosum. This cystic tumor has been observed not only in the parotid gland but also in the submaxillary gland and the mucous glands of the soft palate (Škorpil [40]).

The two tumors of this variety we studied were small, lobulated, cystic, palatal growths of multicentric origin. In a few sections they appeared separated by a thick connective-tissue membrane from the glands (Fig. 4*A*), while other sections revealed extension of the tumor into the glands. The papillae which projected into the cystic cavity were lined with columnar basophilic epithelial cells occasionally supported by

36. Fein, M. W.: Lympho-Epithelioma of Parotid Gland, Am. J. Cancer **40**:434, 1940.

37. Plaut, J. A.: Adenolymphoma of Parotid Gland, Ann. Surg. **116**:43, 1942.

38. Lloyd, O. C.: Salivary Adenoma and Adenolymphoma, J. Path. & Bact. **58**:699, 1946.

39. Kirklin, J. W.; McDonald, J. R.; Harrington, S. W., and New, G. B.: Parotid Tumors: Histopathology, Clinical Behavior, and End Results, Surg., Gynec. and Obst. **92**:721, 1951.

40. Škorpil, F.: Über das Cystadenoma papillare der grossen und kleinen Speicheldrüsen, Frankfurt. Ztschr. Path. **55**:39, 1941.

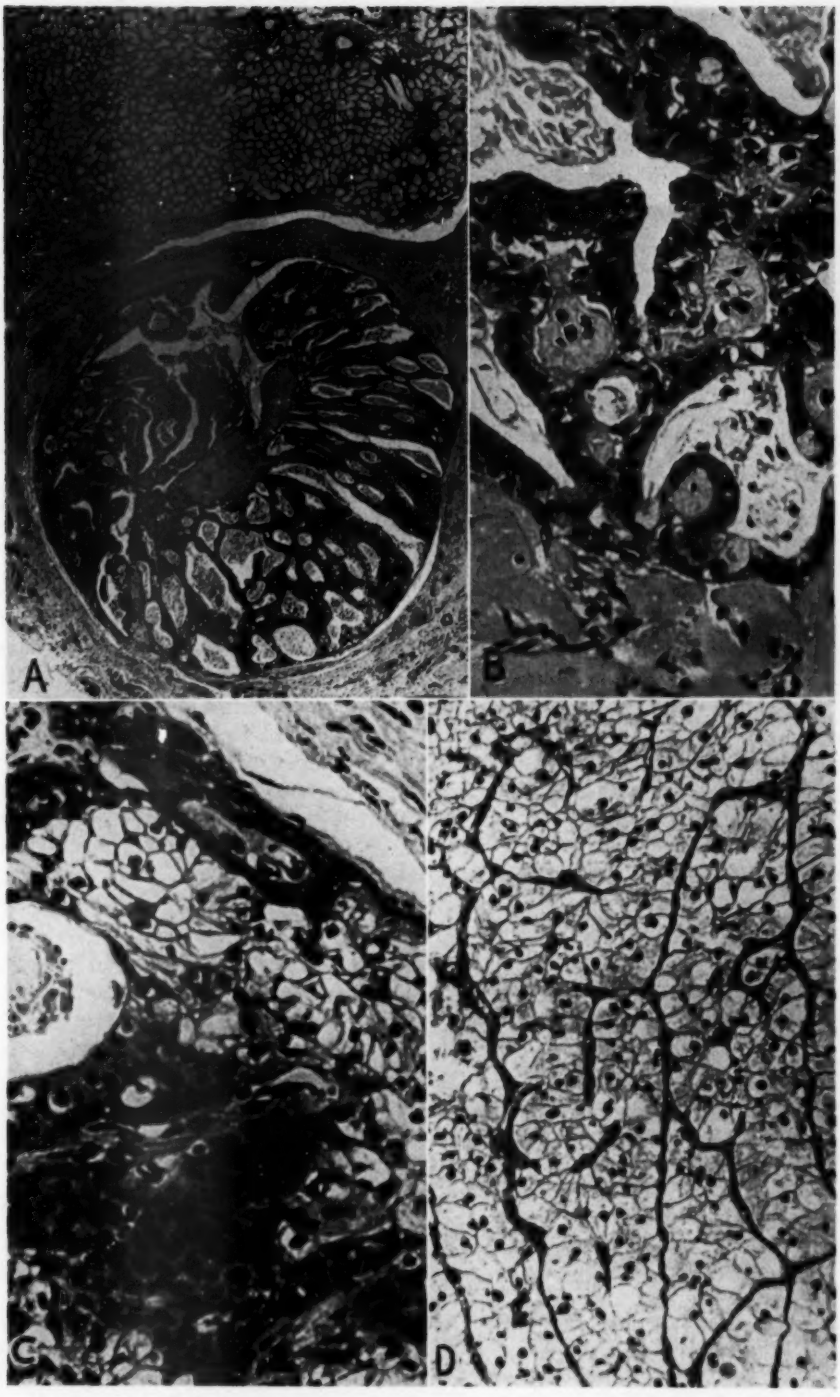


Fig. 4.—*A*, papillary cystadenoma of palatal salivary glands beneath mucous gland. × 30. *B*, area of *A* showing papillary foldings lined by cuboidal epithelium with pyknotic nuclei. × 180. *C*, area of *A* showing transformation of epidermoid cells into "water-clear" cells. × 180. *D*, "clear-cell" variant of bilateral papillary cystadenoma of parotid. × 180.

a layer of cuboidal cells. The nuclei were hyperchromatic. The centers of the projections were occupied by a homogeneous slightly eosinophilic material with nuclei and were occasionally traversed by very delicate capillaries (Fig. 4*B*). The cystic spaces were empty or filled with an oxyphilic substance.

Of interest was the gradual transformation of the cuboidal epithelial cells into water-clear cells which failed to respond positively to mucin, fat, and glycogen stains. This metaplastic change was especially noticeable in the areas where the tumor extended into the mucous glands (Fig. 4*C*). There were foci of epidermoid grouping, also described by Škorpil [40] in salivary papillary cystadenoma; we were therefore inclined to consider the neoplasms as belonging to the mucoepidermoid group, especially since Stewart, Foote, and Becker [2] emphasized the presence of hydropic and swollen cells in benign mucoepidermoid tumors. However, the complete absence of mucous cells in the tumors examined militated against this diagnosis. The absence of lymphoid stroma and the uniformly basophilic lining epithelial cells excluded a papillary cystadenoma lymphomatosum. The cytology of this rare salivary gland neoplasm points to the ductal epithelium as its origin.

Recently Rawson and associates [16] described four tumors which they called "malignant papillary cystadenoma" of the parotid. These tumors had glandular spaces lined with tall columnar eosinophilic cells, but a large amount of mucin was uniformly present in the columnar cells and formed pools in the lumina. This observation indicates that the tumors were of the mucoepidermoid variety rather than of the type being discussed here.

A very interesting but rare variant of papillary cystadenoma of the parotid has been termed "parathyroid-like tumor" by Hückel [41] and Franssen,[42] "hypernephroid" by Škorpil [40] and Rawson and associates,[16] and a mimicry of a "clear-cell renal adenocarcinoma" belonging to the mucoepidermoid type by Stewart, Foote, and Becker.[2] We found the most striking example of this tumor in a 65-year-old man whose right and left parotid glands displayed lobulated tumors which gradually enlarged in the last three years without pain. Removal of these growths showed the left neoplasm well encapsulated and the right one poorly encapsulated.

Microscopic examination revealed identical findings in the bilateral tumors. Anastomosing cords of sharply outlined "water-clear" epithelial cells of various sizes with small hyperchromatic nuclei were supported by a delicate vascular stroma (Fig. 4*D*). The cell membranes had ruptured, and in scattered areas the cells appeared without nuclei. There was no grouping in acini, but ducts were noticeable between the cords and in the clusters of the water-clear cells. Occasionally water-clear cells surrounded wide, empty, vascular spaces. Some areas revealed a dense, hyalinized connective-tissue stroma which contained the hyperchromatic, polymorphic nuclei of a few clear cells. Toward the periphery the epithelial cells showed large granular nuclei with single nucleoli. Some cells with a slightly eosinophilic granular cytoplasm displayed a transformation into clear cells, thereby revealing the origin of the water-clear cells. This observation is in accord with that of Thompson and

41. Hückel, R.: Eine ungewöhnliche Adenomform der Parotis, Verhandl. deutsch. path. Gesellsch. **25**:342, 1930.

42. Franssen, R.: Eine parathyroidea-ähnliche Geschwulst der Ohrspeicheldrüse, Zentralbl. allg. Path. **56**:113, 1932.

Bryant,[30] who demonstrated the transition of oxyphilic granular ductal epithelium into the clear-cell type. However, our study suggests that such a cytoplasmic change is not bound exclusively to oxyphilic cells, for the basophilic acinous and ductal cells of normal and neoplastic parotid glands displayed the same metaplasia. Fat, glycogen, and mucin stains were negative. Stewart, Foote, and Becker [2] observed in about a third of their 26 benign mucoepidermoid tumors such hydropic and swollen clear cells.

The question arises whether this cytoplasmic change indicates a degenerative process or an abnormal differentiation of cells with maintenance of the ability to proliferate. The bilateral occurrence and the steady growth of the observed clear-cell tumor, the integrity of the majority of the nuclei, the highly vascular interductal stroma, and the absence of any necrotic areas pointed to the neoplastic multiplication of the clear cells.

Concerning the origin of these cells, the ductal epithelium and principally the relatively undifferentiated epithelium of the intercalated ducts should be considered. The microscopic features of the bilateral tumors corresponded to those of the "moderately malignant clear-cell carcinoma" of the skin described by Liu.[43] He traced the origin of the clear cells to the basal cells, which are mother cells of the epidermis as well as of the skin appendages. However, while the cells of Liu's [43] tumors contained glycogen, the clear cells of the parotid tumors did not. In this respect they resembled the clear cells (lamprocytes) in the ducts and acini of human mammary glands. Stout and Cooley [43a] recorded a sweat gland epithelioma composed of solid masses of clear, swollen, squamous cells, the polymorphism and nuclear features of which were suggestive of malignancy. The characteristics of the discussed bilateral tumors and the continued good health of the patient did not point to a malignant growth. We considered these bilateral tumors as "clear-cell" variants of papillary cystadenoma of the parotid gland.

7. Mucoepidermoid Tumor.—Though the parotid glands of adults do not contain mucous cells, such cells may occasionally be found in the ducts of a polymorphic adenoma, papillary cystadenoma lymphomatosum, and other parotid neoplasms. However, there are tumors involving predominantly the parotid gland which show mucous and squamous epithelium so conspicuously that Schilling,[44] Masson and Berger,[45] Snellman,[46] Škorpil,[47] and others distinguished them from other salivary gland growths. Masson and Berger [45] termed them "epithéliomas à double métaplasie de la parotide" and Škorpil [47] "the mucous-forming epithelioma of the salivary gland," while Stewart, Foote, and Becker [2] coined the descriptive name "mucoepidermoid tumor of salivary gland." The last-named authors included 45 cases in their

43. Liu, Y.: The Histogenesis of Clear Cell Papillary Carcinoma of Skin, Am. J. Path. **25**:93, 1949.

43a. Stout, A. P., and Cooley, S. G. E.: Carcinoma of Sweat Glands, Cancer **4**:521, 1951.

44. Schilling, F.: Beitrag zur Kenntnis der Parotisgeschwülste, Beitr. path. Anat. **68**:139, 1921.

45. Masson, P., and Berger, L.: Epithéliomas à double métaplasie de la parotide, Bull. Assoc. franc. étude cancer **13**:366, 1924.

46. Snellman, A.: Studien über zylindromatöse Parotisgeschwülste, Arb. Path. Inst. Helsingfors **7**:11, 1933.

47. Škorpil, F.: Über das schleimbildende Epitheliom der Speicheldrüsen, Arch. path. Anat. **305**:661, 1940.

extensive pathological and clinical study. They considered the origin of the mucous cells to be the larger and intermediate ducts. They distinguished a benign and a malignant type. The name they chose is preferable because of the clear emphasis on the two paramount features of the tumor, i. e., mucous cells and clusters and strands of squamous epithelium.

Of the eight specimens we studied, five arose in the parotid gland, two in the soft palate and one in the lower lip. The patients with parotid involvement were between 10 and 35 years old and those with palatal tumors between 25 and 33, while the neoplasm of the lower lip was found in an 18-year-old girl. Three of the five parotid gland tumors occurred in girls aged 10, 12, and 15. These three neoplasms, together with the palatal tumor of a 28-year-old woman, were classified as malignant epidermoid tumors according to the criteria laid down by Stewart and associates.[2] Although the number of our mucoepidermoid tumors is small, it is of some importance that four of eight tumors were microscopically found to be malignant and three of them involved young females.

The tumors were oval and small, averaging 1 to 2 cm. in length. While none of the parotid and palatal growths was well encapsulated, the tumor of the lower lip displayed a definite capsule. With the exception of the neoplasm of the lip, which showed a rather solid structure of epidermoid cords occasionally rimmed with mucus-containing distended cells, the tumors had a multilocular cystic papillary arrangement (Fig. 5*A*). The epithelial foci contained small cystic spaces with infolded projections. The cysts were lined with two or more layers of flattened and cuboidal epithelium, the innermost cells of which in many areas showed a balloon-like expansion by a mucin-positive substance. The outer epithelium was of a squamous type. Some of the cystic spaces originated from dilated ducts; others, we thought, resulted from the breakdown of mucous cells within solid epithelial collections. The presence of the nuclei of ruptured mucous cells verified the latter conclusion. The transformation of columnar and squamous epithelial cells into mucous cells is by no means a degenerative process but is rather a metaplasia of the pluripotential cells of the glandular ducts. Grünwald [48] pointed out recently, "Latent developmental potencies and lability of determination and differentiation are not, as early investigators thought, peculiarities of embryonic cells."

We see further evidence for the wide range of differentiation of the lining cells of the intercalated and striated ducts in the finding of sebaceous glands in mucoepidermoid tumors. Meza-Chavez [6] observed them in 8 of 11 cases and Rawson and Horn [7] in two mucoepidermoid tumors. We found sebaceous elements in only one of the eight mucoepidermoid tumors. This tumor, of the right parotid gland of a 10-year-old girl, was an invasive, fast-growing neoplasm, with poorly differentiated epithelial cells grouped in cords, and with foci of cells of sebaceous gland appearance, rimmed with flattened epithelium (Fig. 5*B*). An area was composed of strands of columnar epithelial cells with light granular cytoplasm and pyknotic nuclei (Fig. 5*C*). The occurrence of sebaceous glands in normal and neoplastic submaxillary and parotid glands has been reported by Hartz,[4] Lee,[5] Meza-Chávez,[20] and Rawson and Horn.[7] Hamperl's [25] conception that an abnormal anlage rather than an acquired

48. Grünwald, P. A.: A Note on the So-Called Undifferentiated and Embryonic Cells, Arch. Path. **36**:190, 1943.

abnormal differentiation gives rise to these sebaceous gland structures should be abandoned. The metaplastic theory is preferable. This opinion appears strengthened by the finding of collections of cells which look like sebaceous cells in craniopharyngioma and suprasellar adamantinoma by Globus and Gang [49] and in the cervix uteri by Donnelly and Navidi.[50]

8. *Adenocarcinoma.*—Of the 13 adenocarcinomas of the salivary glands which we examined, 8 were of the cylindroma type, two showed predominantly papillary cystic structures, and three were adenoacanthomas. Cylindromatous adenocarcinoma of the salivary glands is also called cylindroma. This term should be discarded, since the neoplasm is better labeled on the basis of the morphology of the parenchyma than according to the shape of its stroma.

The eight cylindromatous adenocarcinomas, of which four involved the parotid, two the submaxillary gland and two the palatal mucous glands, displayed roughly the same architecture but differed in cellular details. Features they had in common were various-sized collections of glandular structures, tubules, cords, and solid groups of cells separated by dense fibrous, often hyalinized, strands. However, the shapes of the epithelial cells varied in different areas and tumors. They ranged from well-preserved epithelial cells with distinct normal nuclei to small polymorphic cells with hyperchromatic nuclei resembling basal cells. Epithelial cells grouped in layers encircled rounded spaces of various sizes which were filled with a homogenous material. There were few acini lined with several layers of distinct serous cells, but many rounded cavities proved to be transverse cuts of the cylindrical columns of hyalinized stroma surrounded by parenchymal cells, thereby giving the impression of an alveolar structure. The homogenous substance was either oxyphilic and peripherally rimmed by a sharp, fairly broad, basophilic band or appeared uniformly purple in sections stained with hematoxylin and eosin (Fig. 5*D*). The variable staining reaction is explained by the nature of the material, which is the product of a confluence of hyalinized stroma and secretion of parenchymal cells (Bauer and Fox [15]).

All adenocarcinomas of cylindroma type revealed penetration of the capsule and invasion of nerve sheaths and lymphatics. Two patients with the "basal-cell" type came to autopsy and showed metastases to the lung, liver, and kidney identical with the original parotid tumor.

The two papillary cystadenocarcinomas, one of the parotid gland and the other of the palatal glands, showed small areas of a cylindromatous stroma and in places slightly resembled papillary cystadenocarcinoma of the thyroid. The occasional occurrence of epidermoid features and a few mucous cells was reminiscent of mucoepidermoid tumors, but the paramount histologic structure emphasized the particular type of these tumors. Ahlbom [22] stated that some of these neoplasms which he observed were very malignant.

The three adenoacanthomas of the parotid glands displayed neoplastic proliferation of glandular epithelial cells and keratinizing epithelium. This tumor of the

49. Globus, J. H., and Gang, K. M.: Craniopharyngeoma and Suprasellar Adamantinoma, J. Mt. Sinai Hosp. **12**:220, 1945.

50. Donnelly, G. H., and Navidi, S.: Sebaceous Glands in the Cervix Uteri, J. Path. & Bact. **52**:453, 1950.

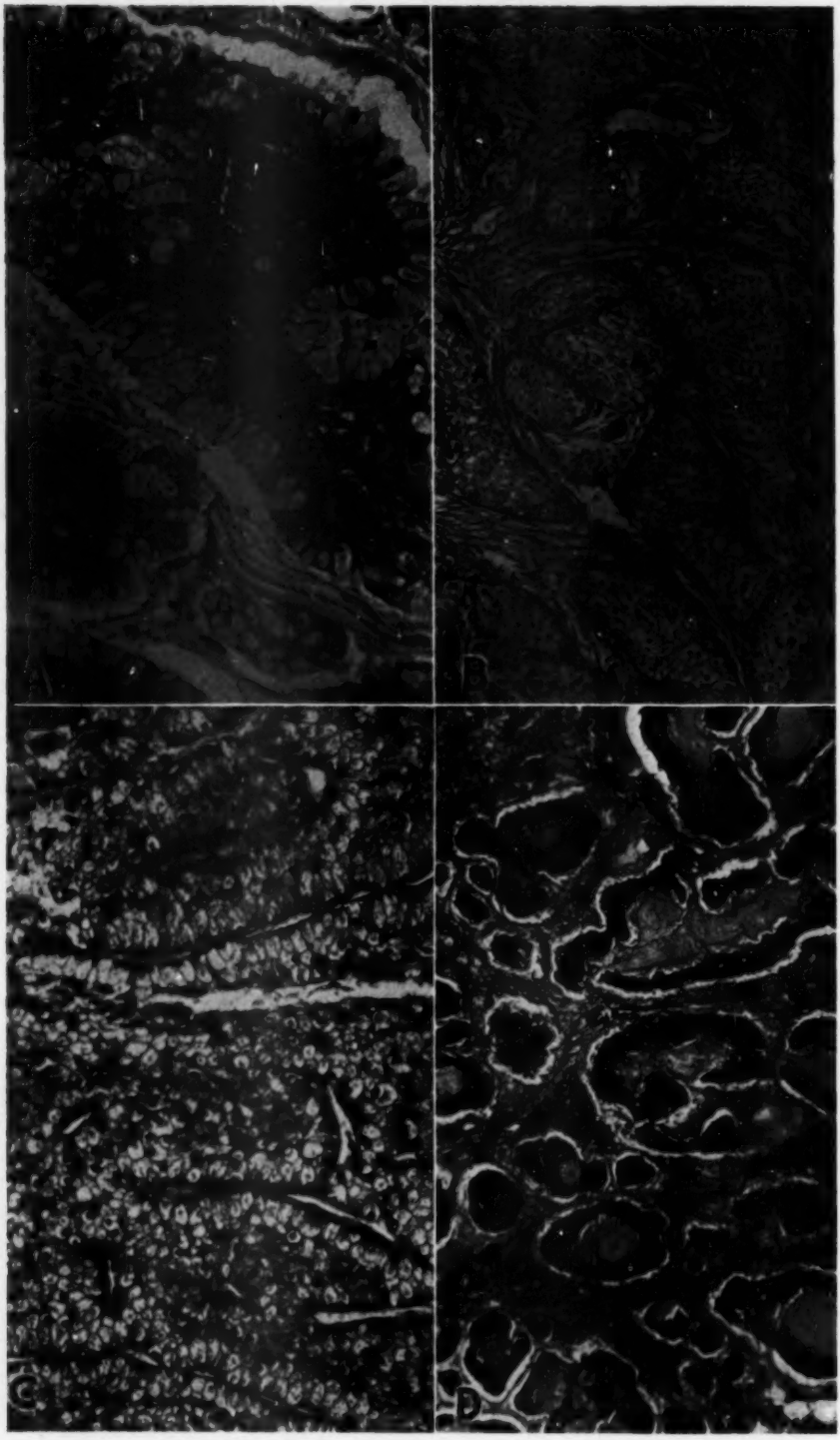


Fig. 5.—*A*, mucoepidermoid tumor of parotid gland, with mucous cells in lining epithelium of papillary projections. × 120. *B*, collections of sebaceous-gland-like structures in mucoepidermoid tumor of parotid gland. × 80. *C*, area of *B* showing papillary projections composed of cells with poorly stained granular cytoplasm and pyknotic nuclei. × 90. *D*, cylindromatous type of adenocarcinoma of parotid gland. × 90.

salivary glands has attracted considerable interest in recent years owing to the work of McPeak and Arons [51] and of McPeak and Warren,[52] who stressed the similarity between adenoacanthoma and papillary adenocarcinoma of the esophagus and the cardiaesophageal junction and adenoacanthoma of salivary glands.

SUMMARY AND CONCLUSIONS

Benign and malignant glandular neoplasms of the salivary glands vary in structure from the common pleomorphic adenoma (so-called "mixed tumors") to true adenoma, oxyphilic granular-cell adenoma, papillary cystadenoma lymphomatosum, lymphomatoid adenoma, papillary cystadenoma, mucoepidermoid tumor, and adenocarcinoma of cylindromatous, papillary, and acanthomatous type. Although these tumors differ in their main structural details, they have a common origin in the lining epithelial cells of the intercalated ducts and less often of the acini. The intercalated ductal epithelium is the matrix of the acinous epithelium and possesses the ability to differentiate into oxyphilic granular, mucous, squamous, sebaceous, and "clear" cells. However, the neoplastic cells may originate from the adult cells of the acini and the larger ducts. The plasticity of the epithelium accounts for the common admixture of features characteristic of different groups. For instance, small areas of pseudocartilage and epidermoid proliferation may be observed in papillary cystadenoma, mucous cells in "polymorphic adenoma," oxyphilic granular cells in papillary cystadenoma lymphomatosum, and so on. However, such occurrence is not significant and does not militate against the specificity of the discussed types.

We consider the so-called "mixed tumor" of the salivary gland to be a pleomorphic adenoma arising from ductal epithelium and exhibiting a peculiar combination of mucoid and pseudocartilaginous material which is a product of secretion, degeneration of epithelial cells, and hyalinization of the stroma. Areas of cartilage and bone may be observed. The polymorphism is accentuated by the participation of myoepithelial cells, which in rare instances dominate the microscopic picture. In these cases a myoepithelial adenoma free from pseudocartilaginous material is formed.

True adenoma of the parotid is an uncommon but distinct neoplasm composed of gland-like structures or cords of the acinous and ductal epithelium.

Oxyphilic granular-cell adenoma (oncocytoma) is composed of large eosinophilic cells with oval or round nuclei with reticular chromatin. The gradual transformation of the basophilic cytoplasm of the ductal epithelium into a granular oxyphilic cytoplasm seems to incite the cells to proliferate and form papillary projections into cystic cavities. Compressed eosinophilic cells with pyknotic nuclei are to be regarded as degenerated. The tumor is of multicentric origin. The unique case of a malignant oxyphilic granular cell adenoma with widespread metastases is discussed.

Papillary cystadenoma lymphomatosum differs distinctly from oxyphilic granular-cell adenoma because of its lymphoid aggregations, although it may display

51. McPeak, E., and Arons, W. L.: Adenoacanthoma of the Esophagus: A Report of One Case with Consideration of the Tumor's Resemblance to So-Called Salivary Gland Tumor, Arch. Path. **44**:385, 1947.

52. McPeak, E., and Warren, S.: Histologic Features of Carcinoma of the Cardia-Esophageal Junction and Cardia, Am. J. Path. **24**:971, 1948.

columnar oxyphilic granular cells in the papillae. Our findings of lymphoid tissue, occasionally large in amount, in a great number of normal parotid glands from persons of various ages led us to the conclusion that this tumor originates in the parotid gland and not in the adjacent lymph nodes.

We propose the name "lymphomatoid adenoma" for a rare neoplasm of the parotid gland. The names under which the few instances of this growth are listed in the literature are misleading. This tumor is neither an adenolymphoma, a term often applied to papillary cystadenoma lymphomatosum, nor a lymphoepithelioma. Lymphomatoid adenoma is a clinical and pathological entity and arises either in the lymphoid aggregates in the parotid gland or in the paraparotid lymph nodes.

Papillary cystadenoma of the salivary glands is a clinical and pathological entity. The occasional occurrence of epidermoid areas in the base of the papillae or in the papillae proper is not sufficient reason to include this tumor in the papillary mucoepidermoid group, for mucin is not detectable. A "water-clear cell" variant is of interest. The arrangement of large hydropic cells with pyknotic nuclei is indistinguishable from that in renal adenocarcinoma. However, unlike "hypernephroma cells," but like the "clear" cells of the mammary glands, these cells do not contain neutral fats, lipids, or glycogen.

In agreement with Stewart and associates,[2] we regard the mucoepidermoid tumor as a clinical and pathological entity. Our finding of three microscopically malignant mucoepidermoid tumors of the parotid in three very young girls is noteworthy. The mucoepidermoid tumors display the widest range of differentiation of epithelial cells into mucous, epidermoid, "clear," and sebaceous cells.

Adenocarcinoma of the salivary glands, found mostly in the parotid and less often in the submaxillary gland, is of cylindromatous, papillary cystic, or adenoacanthomatous type.

LOCALIZING PROPERTIES OF ANTI-RAT-KIDNEY SERUM PREPARED IN DUCKS

DAVID PRESSMAN, Ph.D.
LEONHARD KORNGOLD, Ph.D.
NEW YORK
AND
WALTER HEYMANN, M.D.
CLEVELAND

HEYMANN, Lund, and Hackel [1] have recently shown that anti-rat-kidney sera prepared in either rabbits or ducks produce the nephrotic syndrome in rats. There is a difference in the actions of the sera, since symptoms developed immediately when rabbit antisera were used while with duck antisera a lag period of 3 to 20 days preceded the onset of kidney damage.[2] There is also a higher incidence of uremia with the duck serum.[1]

Present theories on the action of nephrotoxic antikidney sera postulate an initial localization of antibodies in the kidney. That this actually happens when antikidney sera prepared in rabbits are injected into rats was shown by means of radioactive tracer techniques.[3]

The present report compares the localizing properties of nephrotoxic anti-rat-kidney sera prepared in ducks and in rabbits.

METHODS

The rabbit and duck anti-rat-kidney sera were prepared as described previously.[4] The globulin fractions of both antisera and normal sera were obtained by salting with ammonium sulfate at a final concentration of 1.75M. The globulin fractions were iodinated and assayed for localizing activity according to the procedure described previously.[5] Sherman rats were used for assay.

This investigation was supported in part by the Atomic Energy Commission and by the National Institutes of Health, United States Public Health Service.

From the Sloan-Kettering Institute for Cancer Research (Dr. Pressman and Dr. Korngold) and the Department of Pediatrics, Western Reserve University, School of Medicine (Dr. Heymann).

1. Heymann, W.; Lund, H. Z., and Hackel, D. B.: J. Lab. & Clin. Med. **39**:218, 1952.

2. That rabbit antikidney sera might be different from duck antikidney sera was already evident from the work of Masugi. (*a*) Masugi, M.: Beitr. path. Anat. **91**:82, 1933; **92**:429, 1934. He prepared duck antisera against rabbit kidneys and rabbit antisera against rat kidneys. Consequently it is not clear whether the differences observed were due to the species which provided the sera or to the species in which kidney damage was produced. Other investigators who used avian antiserums have also reported lag periods—for example, (*b*) Kay, C. F.: Am. J. M. Sc. **204**:483, 1942—while investigators using rabbit sera observed immediate effects—for example, (*c*) Heymann, W., and Hackel, D. B.: J. Lab. & Clin. Med. **39**:429, 1952, and (*d*) Lippman, R. W.; Marti, H. U., and Jacobs, E. E.: Proc. Nat. Acad. Sc. **38**:260, 1952.

3. Pressman, D., and Keighley, G.: J. Immunol. **59**:141, 1948.

4. Both antiserums were nephrotoxic. We are indebted to Dr. Beatrice Seegal, of the College of Physicians and Surgeons, Columbia University, for determining the nephrotoxic properties of the rabbit antiserum. Footnotes 1 and 3.

(Footnotes continued on next page)

COMMENT

The results are presented as the per cent of the injected radioactivity which localized in 1 gm. of tissue (Table). It can be seen that antikidney sera, prepared in either the duck or the rabbit, localized to a greater degree in kidney (and in liver) than the control normal sera.

The different physiological activities of the duck and rabbit sera, therefore, cannot be attributed to any inability of duck antikidney serum to localize in the rat kidney.

According to Kay,[2b] the mechanism for the production of kidney damage in rabbits by duck antikidney serum is the following: (*a*) Duck antikidney antibodies localize in the rabbit without a physiological effect; (*b*) the rabbit subsequently produces antibodies against duck serum; (*c*) these antibodies combine with the duck serum which had localized in the kidney, thereby producing kidney damage. Since it takes several days to produce antibodies in the rabbit, Kay's theory takes care of the lag period observed when duck sera are used. It does not account for the very rapid response of the rat when rabbit antikidney sera are used.[2c,d] However, it is possible to explain the differences by a mechanism such as Kay proposed but also involving complement fixation. Since it is known that complement is

*Localization of Rabbit and Duck Anti-Rat-Kidney Antibodies in the Rat**

Globulin Fraction From	Rats, No.	% Localized †	
		Kidney	Liver
Duck, normal serum	3	0.04	0.06
Duck, antikidney serum	3	0.25	0.15
Rabbit, normal serum	6	0.09	0.10
Rabbit, antikidney serum	3	0.42	0.19

* Each rat was inoculated with 0.5 mg. of globulin fraction (2×10^6 count/minute) and assayed three days later. Values are averages with mean deviations of less than 10%.
† Per cent of the injected radioactivity which localized in 1 gm. of tissue per 100 gm. rat.

essential to the production of cellular damage, such as lysis of erythrocytes and bacteria by antibodies, it may be that complement fixation is also required for the kidney damage produced by antikidney antibodies.[6] Rabbit antibodies fix complement, while duck antibodies do not.[7] On this basis, rabbit antiserum would be immediately effective, while duck antiserum would not. Only after the rats produce antibodies to the injected duck serum can complement be fixed in the kidney and damage ensue.

SUMMARY

It is shown that kidney-localizing antibodies are present in nephrotoxic anti-rat-kidney sera prepared in ducks as well as in rabbits.

5. Pressman, D., and Eisen, H. N.: J. Immunol. **64**:273, 1950. Pressman, D., and Sherman, B.: Ibid. **67**:15, 1951.

6. Physiological damage to a cell does not necessitate the action of antibodies specific to the cells. Investigators have shown that the reaction of antiprotein antibody with cells coated with the homologous protein in the presence of complement causes lysis (for example, Adler, F. L.: Proc. Soc. Exper. Biol. & Med. **74**:561, 1950).

7. Izumi, F.: Folia endocrinol. **16**:48, 1940. Rice, G. E.: Canad. J. Comp. Med. **11**:236, 1942. A. B. Stavitsky, and W. Heymann (unpublished data) have found that rabbit antikidney serum fixes circulating complement within less than 30 minutes after injection into the rat.

News and Comment

ANNUAL SESSION OF THE AMERICAN MEDICAL ASSOCIATION

New York, June 1-5, 1953

The following portion of the program of the American Medical Association Scientific Session relating to pathology and physiology will be of interest to readers of this Journal.

SECTION ON PATHOLOGY AND PHYSIOLOGY

BAROQUE ROOM, BELMONT PLAZA HOTEL

Tuesday, June 2—9 a. m.

The Comparative Physiology of the Kidney — Homer W. Smith, New York

Discussion to be opened by Robert F. Pitts, New York

Metabolism of Uric Acid and Its Relation to Gout — William S. Hoffman, Chicago

Discussion to be opened by Alexander B. Gutman, New York

Chemical Inactivation of Viruses in Blood and Plasma

Frank W. Hartman, George Magnum, A. R. Kelley, and Gerald Lo Grippo, Detroit

Discussion to be opened by Bettylee Hampil, Glenolden, Pa.

Immunohematologic Studies in Hemolytic Anemia — Israel Davidsohn, Chicago

Newer Approaches to the Study of Hemophilia and Hemophilia-like Hemorrhagic States

K. M. Brinkhous, R. D. Langdell, G. D. Penick, J. B. Graham, and R. H. Wagner, Chapel Hill, N. C.

Discussion on papers of Dr. Davidsohn and Drs. Brinkhous et al. to be opened by Matthew H. Block, Chicago, and Max M. Strumia, Bryn Mawr, Pa.

Factors Determining Arterial Blood Pressure — Lysle H. Peterson, Philadelphia

Discussion to be opened by J. Earl Thomas, Philadelphia

Postemphysematous or Phrenic Hypertension: An Analysis of 75 Cases of Chronic Asthma Complicated by Emphysema

Charles F. Geshickter and Antoinette Popovici, Washington, D. C.

Discussion to be opened by Albert H. Andrews Jr., Chicago

Wednesday, June 3—9 a. m.

BUSINESS MEETING
ELECTION OF OFFICERS

Chairman's Address: Medical Technology and Its Relation to Physiology and Pathology — Lall G. Montgomery, Muncie, Ind.

Blood Volume: Evaluation of Methods and Interpretation of Results

M. I. Gregersen, New York

Control of Trichinosis by Irradiation of Pork — S. E. Gould, Eloise, Mich.

Discussion to be opened by Henry J. Gomberg and Frank H. Bethell, Ann Arbor, Mich., and Vernon B. Link, Atlanta, Ga.

Diffuse Interstitial Fibrosis of the Lungs

ALFRED GOLDEN and THEODORE T. BRONK, Buffalo

Discussion to be opened by MAURICE N. RICHTER, New York, and MILTON G. BOHROD, Rochester, N. Y.

Atypical Adenoma of the Thyroid JOHN B. HAZARD and REX KENYON, Cleveland

Distribution of Thyroid Cancer in Thyroid Glands with Discussion of Clinical Significance in Treatment

WILLIAM O. RUSSELL, R. LEE CLARK JR., and CANDIDO DE OLIVEIRA, Houston, Texas

Discussion on papers of DRS. HAZARD and KENYON and DRS. RUSSELL, CLARK and DE OLIVEIRA to be opened by W. A. D. ANDERSON, Milwaukee

Histologic Classification and Prognosis of Hydatidiform Moles

HUGH G. GRADY, Gaithersburg, Md., and CHARLES I. BRYANS JR., Augusta, Ga.

Discussion to be opened by GEORGE MILLES, Chicago

A Hyperglycemic Agent in Pancreatic Extracts: A Possible Factor in Certain Types of Diabetes I. J. PINCUS, Philadelphia

Discussion to be opened by JOSEPH T. BEARDWOOD, Philadelphia, and RACHMIEL LEVINE, Chicago

Thursday, June 4—9 a. m.

JOINT MEETING WITH SECTION ON GASTROENTEROLOGY AND PROCTOLOGY IN THE GRAND BALLROOM OF THE NEW YORKER

Polyps of the Colon and Rectum NEIL W. SWINTON, Boston

Discussion to be opened by MARIE ORTMAYER, Chicago; JAY M. GARNER, Winnetka, Ill., and ROBERT A. SCARBOROUGH, San Francisco

Pathology of Regional Ileitis and Ulcerative Colitis

SHIELDS WARREN and SHELDON C. SOMMERS, Boston

Discussion to be opened by JOSEPH B. KIRSNER, Chicago; RICHARD B. CATTELL, Boston, and BURRILL B. CROHN, New York

The Role of the Adrenal Steroids in the Pathogenesis of Peptic Ulcer

SEYMOUR J. GRAY, COLIN G. RAMSEY, ROBERT W. REIFENSTEIN, and JOHN A. BENSON, Boston

Discussion to be opened by HARRY SHAY, Philadelphia; DAVID J. SANDWEISS, Detroit, and MORTON I. GROSSMAN, Chicago

The Two-Component Mucous Barrier: Its Role in Protecting the Gastroduodenal Mucosa Against Peptic Ulceration FRANKLIN HOLLANDER, New York

Discussion to be opened by J. EARL THOMAS, Philadelphia

Enterobius Vermicularis Granuloma of the Appendix Vermiformis

JOHN R. SCHENKEN, Omaha; FRANCIS C. COLEMAN, Des Moines, Iowa, and EMMA S. MOSS, New Orleans

The Management of Enterobiasis WILLIAM J. ECKERLE, Rolling Hills, Calif.

Discussion on papers of DRS. SCHENKEN, COLEMAN, and MOSS, and ECKERLE to be opened by EMMA S. MOSS, New Orleans

The Oral Use of Hydrocortisone (Compound F) in the Treatment of Sprue

DAVID ADLERSBERG, HENRY COLCHER, and CHUN-I WANG, New York

Discussion to be opened by PERRY J. CULVER, Boston

NOTICE OF MEDICAL MEETINGS

Several meetings of interest to pathologists will be held at the Texas Medical Center, Houston, Texas, on May 15 and 16.

These will include the "Seventh Annual Symposium on Fundamental Cancer Research," of The University of Texas M. D. Anderson Hospital for Cancer Research. One half-day will be devoted to selected papers on the subject of "New Methods for the Study of Cells." The Cancer Pathology and Radiology Conference of The University of Texas M. D. Anderson Hospital and Postgraduate School of Medicine will be on "Tumors of Lung and Pleura." A meeting of the South Central Section of the College of American Pathologists will co-participate in the Symposium and Pathology Conference. There will be papers of general interest to pathologists. Dr. Robert C. Mellors, department of cytochemistry, department of experimental pathology, Sloan-Kettering Institute, Memorial Hospital, New York, is arranging the session on cytological studies. Dr. David A. Wood, professor of oncology (pathology) and director of the cancer research institute of the University of California Medical School, and Dr. W. Edward Chamberlain, professor of radiology, Temple University Hospital, Philadelphia, will conduct the pathology and radiology conference.

The fourth Bertner Foundation Lecture will be given by Dr. Charles C. Huggins, professor of surgery, University of Chicago School of Medicine, at the banquet on May 15; his subject will be "The Control of Human Cancer by Hormonal Methods." All meetings will be held at The Shamrock. Further information may be obtained from William O. Russell, M.D., 2310 Baldwin Street, Houston 6, Texas.

New Appointment for Dr. Forbus.—Wiley D. Forbus, M.D., Chief of Service, Department of Pathology, Duke University School of Medicine, has been appointed Consultant to the Division of Biology and Medicine, United States Atomic Energy Commission.

American Goiter Association.—The 1953 Annual Meeting of the American Goiter Association will be held in the Drake Hotel, Chicago, May 7-9. The program will include papers dealing with goiter and other disorders of the thyroid gland.

Books

Reaction to Injury: Volume II. The Reactions of Submission and Adaptation and the Disease Entities Arising Out of Their Elaboration. By Wiley D. Forbus, M.D., Professor of Pathology, Duke University; Pathologist, the Duke Hospital. Price, $20.00. Pp. 1,110, with 836 illustrations and with 54 color plates. Williams & Wilkins Company, Mount Royal and Guilford Aves., Baltimore 2, 1952.

Ever since Forbus' impressive work, "Reaction to Injury: Vol. I. Pathology for Students of Disease," appeared in 1943, many have looked forward with interest to the publication of the promised second volume. After nine years this has appeared. It is 313 pages longer and almost 2 lb. heavier than Volume I. Even a casual survey of this new volume enables one to appreciate the enormous amount of labor that has gone into its preparation and to understand why it was so long in gestation. But the waiting was worth while. In the author's opinion, "The two volumes include all the materials usually considered essential for a textbook of general pathology." They also contain much that could be classified as clinical medicine.

In the preparation of these volumes Dr. Forbus departed entirely from the old conventional treatment of the subject. His interest is in "the whole individual, a mass of integrated, reacting cells, tissues, organs and systems." The panorama of pathology here unrolled is based upon a preconceived plan that has been rigidly adhered to throughout. Briefly, this plan is as follows: "The essential element of disease is considered to be the *reaction* of the individual, in the last analysis, the reaction of the individual cells that compose the tissues, to agents and influences comprising the environment. The individual is considered to be capable of reacting in only three essential ways, 1) by resisting, 2) by submitting and 3) by effecting an adaptation. These three reactions, therefore, are taken to be the basis of all disease, and the recognized disease entities are considered to be the expression of the elaboration of one or several of these reactions." This general concept of disease was developed in the first 120 pages of Volume I, the remainder of which was devoted to a detailed study of reaction by resistance.

In Volume II the author elaborates his concepts of reaction by submission and adaptation. The first 870 pages of Volume II are devoted to "the submissive reaction to injury and the disease entities arising therefrom." Section 1 is concerned with "disturbances in function and metabolism of cells and tissues." After a chapter on the normal and the abnormal cell, atrophy, necrosis, pigmentations, calcification, etc., there follow seven chapters dealing with "certain common forms of intoxication of extrinsic origin," which constitute an excellent summary of the basic principles of toxicology. In the last chapter in this section, disturbances due to local changes in the circulation—hyperemia, edema, hemorrhage, thrombosis, embolism, and infarction —are presented from the standpoint of their effect on the function and metabolism of the cells and tissue affected by these processes.

Section 2, "Disturbances in Organic and Systemic Function and Metabolism," is divided into three subsections. Subsection 1 deals with disturbances in structure, function, and metabolism related to the vitamins and other essential nutritional substances, including fats and lipoids (arteriosclerosis, lipodystrophies), and proteins (gout, amyloidosis, etc.). Subsection 2 is concerned with "disturbances in function and metabolism related to the formation of and injury to the oxygen-carrying and other elements of the blood" and includes the anemias and blood dyscrasias. Subsection 3 is a comprehensive discussion of disordered function and metabolism related to the endocrine glands.

Section 3, "Disturbances in Function and Metabolism Related to the Processes of Growth and Development," is divided into two subsections. Subsection 1 is concerned with disturbances (malformations and monstrosities) in the process of growth and development during the prenatal period. It may seem strange, but it is basically logical that malformations of the heart and great vessels are reserved for Part IV, which deals with reaction by adaptation. Subsection 2 deals with disturbances related to the process of growth and development during the postnatal period (including leukemias, lymphosarcoma, agranulocytosis, etc.) and contains one chapter

of 75 pages on tumors. The author makes no apology for the fact that, for some readers, the diagnosis of individual tumors may be "disappointingly brief." He is only interested in the basic principles of neoplasia.

In Part IV, which is divided into four sections, the author considers the "reaction to injury through adaptation." He meets frankly the philosophic difficulties in the age-old conflict between teleologic and mechanistic conceptions of natural phenomena. He has adopted a "strictly utilitarian attitude" toward this problem. He considers adaptation "simply as an adjustment in relationships, and this may or may not be specifically in the interest of either the cell or the body. . . . Pathologic adaptation occurs strictly within what one may call the normal physiologic and anatomic range of normal cells." In this sense, therefore, adaptation differs fundamentally from Selye's "Adaptation Syndrome."

Section 1 of Part IV deals with general considerations of the principles of adaptation and with regeneration of tissues and the healing of wounds. Section 2, "Adaptive Reactions Following Injury to the Circulatory System," has chapters on adjustments resulting from cardiac obstruction to the flow of blood and on adjustments associated with obstructive injury to the blood and lymph vessels. In Section 3, the adjustments following obstruction within the respiratory tract are discussed. Adaptive reactions following obstruction to the passage of body fluids from and through hollow viscera constitute Section 4. In this section there are chapters on adjustments to obstruction of the ducts of glands of external secretion, obstruction of the gastrointestinal tract and the urinary tract, and obstruction of the flow of cerebrospinal fluid.

Since these two volumes, particularly Volume II, differ so fundamentally from the usual textbook of pathology, it has seemed necessary to present the above extensive outline of the contents, in order to furnish an adequate idea of what the author has done. He has attempted (1) to provide an "easily grasped conception of the whole problem" of pathology, (2) to show that "what might at first seem to be hopelessly confused biological, chemical and physical data can be sorted out and arranged" in an orderly manner, and (3) to center attention upon "broad relationships and general principles rather than upon isolated, factual and often deceivingly dramatic details." The result is a very stimulating book.

A rigid outline or pattern has its disadvantages. One would like to know the reasoning that caused the author to place some subjects in the particular section where they are found. For example, endemic and epidemic hepatitis are included in the general section on "reaction by submission," although he discusses the reparative (resistive? adaptive?) processes in those patients who recover. Thromboangiitis obliterans, periarteritis nodosa, and eclampsia are included in Part IV on adaptations.

The author's style may be described as "easygoing." It reminds one of a remark of Dr. J. George Adami in the preface to his "Principles of Pathology." "I found," said he, "that I could easily remember the matter in work . . . in which there was a reasoned treatment of the subject. . . . I saved time and gained knowledge by reading my subject at large." Adami would have thoroughly enjoyed these two volumes by Dr. Forbus. The author uses many simple words and phrases that add pleasure to reading. For example, a sensitized person exposed to a second injection of the sensitizing material "breaks out into a multitude of small elevated red bumps." The 836 illustrations (54 in color) are generally excellent, and most of them are from the author's own material. Here also he had a very definite plan based upon the feeling that illustration for decoration alone is unjustifiable. "Full and descriptive legends have been employed because experience has shown that the uninitiated most often sees only what is pointed out to him." These legends are among the unique features of this work.

The format of the book is excellent. It is printed in large, clear type with two columns to the page, which makes for easy reading. One unpleasant feature is the high gloss to the paper, that necessitates having the light properly placed in order to avoid an unpleasant glare. In the bibliography, only those contributions are listed which have been found especially useful. There is an adequate index of 20 pages with 3 columns to the page. Only a few typographical errors were discovered.

Every pathologist and physician who read Volume I will want to read this second volume. Together they furnish a remarkably comprehensive exposition of the general principles of the whole field of pathology. The pathologist may not find any new facts, but he will find a new and stimulating viewpoint from which to think of pathology—and this is much to be desired.

Correlative Cardiology: An Integration of Cardiac Function and the Management of Cardiac Disease. By Carl F. Shaffer, M.D., F.A.C.P., Associate Professor of Clinical Medicine, Baylor University College of Medicine, and Don W. Chapman, M.D., F.A.C.P., Associate Professor of Medicine, Baylor University College of Medicine. Price, $9.50. Pp. 525, illustrated. W. B. Saunders Company, 218 W. Washington Sq., Philadelphia 5, 1952.

Carbohydrate Metabolism: A Symposium on the Clinical and Biochemical Aspects of Carbohydrate Utilization in Health and Disease. Edited by Victor A. Najjar. Price, $4.00. Pp. 134, with numerous illustrations. The Johns Hopkins Press, Homewood, Baltimore 18, 1952.

Carbohydrate Metabolism: Correlation of Physiological, Biochemical and Clinical Aspects. By Samuel Soskin, M.D., Director of the Medical Research Institute, Michael Reese Hospital; Dean of the Michael Reese Hospital Postgraduate School; Associate Professor of Medicine, Northwestern University, and Rachmiel Levine, M.D., Director of Metabolic and Endocrine Research, Michael Reese Hospital; Professorial Lecturer in Physiology, University of Chicago. Revised edition. Price, $8.50. Pp. 346, with 75 illustrations and 42 tables. University of Chicago Press, 58th St. and Ellis Ave., Chicago 37, 1952.

Microscopic Histochemistry: Principles and Practice. By George Gomori, M.D., Professor of Medicine, University of Chicago. Price, $6.00. Pp. 273, with 1 illustration and 6 tables. University of Chicago Press, 58th St. and Ellis Ave., Chicago 37, 1952.

The merit of Gomori's "Microscopic Histochemistry" is the clear, practical description of methods by an author evidently well versed in most of them and responsible for the introduction and popularization of many of them. Histochemical methods involve both tissue and reagent; the preparation of the one and the choice of the other lead to infinite possibilities of modification, as the literature will attest. The reactions given here appear to be well chosen, and while applicable to routinely fixed tissues (using formaldehyde, acetic acid, alcohol, acetone). would, in the main, be adaptable for such techniques as freeze-drying. Enzyme methods are well described, and the tables of substrates and pH optima should be most useful. The list of buffers will be appreciated by anyone who has tried to find them in the literature. Rationales of the methods are briefly and reasonably discussed, except perhaps for the criticism of the 40-year-old work of Unna, which seems a little passionate. Of more immediate interest is the nonapplicability of every test (or battery of tests) for the histochemical demonstration of ketosteroids. Within its restricted branch of histochemistry, this book can be recommended to the pathologist seeking special tests for his material.

The Principles and Methods of Physical Diagnosis: Correlation of Physical Signs with Physiologic and Pathologic Changes in Disease. By Simon S. Leopold, M.D., Associate Professor of Clinical Medicine, School of Medicine and Graduate School of Medicine, University of Pennsylvania; Director of the Teaching of Physical Diagnosis, School of Medicine, University of Pennsylvania; Chief of the Thoracic Clinic, Hospital of the University of Pennsylvania. With a Chapter on Sounds from the Thorax: Acoustic Principles. By S. Reid Warren, Jr., Sc.D., in E.E., Professor of Electrical Engineering, the Moore School of Electrical Engineering, University of Pennsylvania. Price, $7.50 Pp. 430, with 390 illustrations and 19 color plates. W. B. Saunders Company, 218 W. Washington Sq., Philadelphia 5, 1952.

Physician's Handbook. By John Warkentin, and J. D. Lange. Seventh edition. Edited by Marcus A. Krupp, M.D., assistant clinical professor of medicine, Stanford University School of Medicine; director, Palo Alto Medical Research Foundation, Palo Alto; Norman J. Sweet, M.D., assistant professor of medicine, University of California Medical School, San Francisco; Ernest Jawetz, Ph.D., M.D., associate professor of bacteriology and lecturer in medicine and pediatrics, University of California Medical School, San Francisco, and Charles D. Armstrong, M.D., clinical instructor in medicine, Stanford University School of Medicine. Price, $2.50. Pp. 380, with numerous illustrations and tables. Lange Medical Publications, Los Altos, Calif., 1952.

Leverens glukoseafgift under insulin-paavirkning. By Keld Steincke. Price, not given. Pp. 206, with 45 illustrations. Dansk Videnskabs Forlag A/S, Copenhagen, 1951.

Lehrbuch der Röntgendiagnostik. By H. R. Schinz, W. E. Baensch, E. Friedl, and E. Uehlinger. Part 8: Innere Organe. Price, 96 German marks. Pp. 454, with 559 illustrations. Georg Thieme, Diemershaldenstrasse 47, (14a) Stuttgart-O; agents for U. S. A.: Grune & Stratton, Inc., 381 Fourth Ave., New York 16, 1952.

Electrocardiography in Practice. By Ashton Graybiel, M.D., Captain, Medical Corps, United States Navy; director of research, United States Naval School of Aviation Medicine, Pensacola, Fla.; Paul D. White, M.D., executive director, National Advisory Heart Council; consultant in medicine, Massachusetts General Hospital, Boston; Louise Wheeler, A.M., executive secretary, the Cardiac Laboratory, Massachusetts General Hospital, and Conger Williams, M.D., instructor in medicine, Harvard Medical School; associate physician, Massachusetts General Hospital. Price, $10.00. Pp. 378, with 294 illustrations. W. B. Saunders Company, 218 W. Washington Sq., Philadelphia 5; 7 Grape St., Shaftesbury Ave., London, W.C. 2, 1952.

Standard Values in Blood. Edited by Errett C. Albritton, A.B., M.D., Fry Professor of Physiology, George Washington University School of Medicine, Washington, D. C. Price, $4.50. Pp. 199, with 101 tables. Prepared under the direction of the committee on the Handbook of Biological Data, American Institute of Biological Sciences, The National Research Council. W. B. Saunders Company, 218 W. Washington Sq., Philadelphia 5; 7 Grape St., Shaftesbury Ave., London, W.C. 2, 1952.

Shock Syndrome. By John Scudder, M.D., and twenty-five other authors. Price, $3.75. Pp. 375, with numerous illustrations and tables. Annals of the New York Academy of Sciences, Volume 55, Article 3. The New York Academy of Sciences, 2 E. 63d St., New York 21, 1952.

The Chick Embryo in Biological Research. By David A. Karnofsky, M.D., and twenty-seven other authors. Price, $4.00. Pp. 400, with numerous illustrations and tables. Annals of the New York Academy of Sciences, Volume 55, Article 2. The New York Academy of Sciences, 2 E. 63d St., New York 21, 1952.

Morbus Alzheimer and Morbus Pick: A Genetic, Clinical and Patho-Anatomical Study. By Torsten Sjögren, Hakon Sjögren, and Åke G. H. Lindgren. Supplementum 82, Acta psychiatrica et neurologica scandinavica. Price, 25 Swedish crowns. Pp. 152, with 12 illustrations and 14 tables. Ejnar Munksgaards Forlag, Nørregade 6, København, K, 1952.

Photosensitization in Diseases of Domestic Animals: A Review. By N. T. Clare, M.Sc., Ruakura Animal Research Station, Department of Agriculture, Hamilton, New Zealand. Review Series No. 3 of the Commonwealth Bureau of Animal Health. Price, 7/6 d. Pp. 58, with 8 illustrations. Commonwealth Agricultural Bureaux, Farnham Royal, Bucks, England, 1952.

Atlas of Tumor Pathology: Section IV, Fascicle 16, Tumors of the Carotid Body and Related Structures (Chemoreceptor System). By Philip M. LeCompte, M.D., Pathologist, Faulkner Hospital, Boston; Instructor in Pathology, Harvard Medical School. Price, $0.45. Pp. 40, with 23 illustrations and 1 color plate. Published by the Armed Forces Institute of Pathology, under the auspices of the Subcommittee on Oncology of the Committee on Pathology of the National Research Council, 2101 Constitution Ave., N. W., Washington 25, D. C., 1951.

This brief fascicle is splendidly concise, excellently illustrated, and remarkably complete. In a brief compass it covers the normal chemoreceptor system and the tumors that arise therefrom. The pathological features are illustrated by photomicrographs of high excellence. In the matter of treatment a strong plea is made for conservative management once the diagnosis has been made. There is a substantial and useful list of references. The volume can be highly recommended.

Viral and Rickettsial Infections of Man. Edited by Thomas M. Rivers, M.D., director of the Hospital of the Rockefeller Institute for Medical Research. Second edition. Price, $7.50. Pp. 719, with 90 illustrations including 7 plates in color, and 25 tables. J. B. Lippincott Company, 227-231 S. 6th St., Philadelphia 5; Aldine House, 10-13 Bedford St., London, W.C. 2; 2083 Guy St., Montreal, 1952.

The first edition of this unique and valuable book was published four years ago, and the high standards set at that time are well maintained in the second edition. The cooperation of 30 authors, each thoroughly familiar with his particular field, has resulted in discussions which are authoritative and critical. Dangers inherent in multiple authorship, such as lack of uniformity in style, differences in fundamental viewpoints and undue emphasis on individual work, have been admirably avoided. Excellent balance and correlation have been achieved between basic theoretical knowledge and practical clinical aspects. As a result of this, the book is of value not only to physicians and students who wish to acquire up-to-date knowledge of viral and rickettsial infections but also to investigators who are seeking profitable fields for further experimental studies of this important and interesting group of diseases.

Viruses and rickettsiae are obligate intracellular parasites, which have not been cultivated in cell-free media because they are, in variable degrees, metabolically dependent on their host cells. The first 170 pages of this book deal with important concepts concerning the nature of viruses and rickettsiae, their biologic properties, and the unique and ingenious methods of study which have led to advances in knowledge. Twenty pages are wisely devoted to studies of bacterial viruses from which so much of our basic knowledge of host-parasite relationships has been acquired. Most of the remainder of the book consists of detailed discussions of the common and important viral and rickettsial diseases of man, using the vertical approach. In the last 13 pages "Infections of Minor Importance" are considered. It might be remarked that certain conditions considered here, such as cat-scratch disease and generalized salivary gland virus infection, are not strictly speaking of minor importance but rather have not yet assumed their true importance because of incomplete study and recognition.

Diseases of reasonably certain viral etiology which are omitted from the book are hepatoadrenal necrosis of infants, chronic "inclusion encephalitis," giant cell pneumonia, epidemic pneumonia of infants as described by Adams and his co-workers, and a type of infantile pneumonia described by Goodpasture. At present these diseases are diagnosed only after postmortem study, by finding the characteristic inclusion bodies. They are being diagnosed by pathologists with increasing frequency, however, and their inclusion in subsequent editions of this book would be advantageous, since it would call attention to important unsolved problems in which pathologists, by saving tissues in the frozen state and subsequently recognizing inclusions, are destined to initiate progress.

Notable improvements over the first edition include new chapters on the recently discovered Coxsackie viruses, viral hemagglutination, interference between animal viruses, and diagnostic methods, as well as a careful revision of all subjects considered, in the light of recent evidence. The low cost, made possible only because of subsidy by the National Foundation for Infantile Paralysis, remains unchanged. This inexpensive and well-edited book might well serve as a model for similar group endeavors in other fields, which are occupying an increasingly important position in modern cooperative medical research and teaching.

CORRECTION

In the article by Drs. Ruby M. Collister, J. Dankmeijer, H. A. Snellen, and W. H. van der Wel entitled "Postmortem X-Ray Studies of Congenital Malformations of the Heart," published in the January issue, page 31, Dr. van der Wel's degree was incorrectly given as M.D., instead of B.M. The fourth sentence of the last paragraph of the report of Case 4 should read, "In addition, however, this procedure also revealed the existence of an interatrial septal defect, which had not been seen on our films." The fifth sentence before the Summary should read, "Had this in fact existed, then the filling of the left ventricle following injection into the right ventricle could be explained only by the presence of an interventricular septal defect."

PARAGON STAINS **PARAMOUNT QUALITY**

PARAGON STAINING SOLUTIONS

For Tissue Sections

Dependable—Today; Tomorrow; Every Day

With Paragon Staining Solutions you obtain superbly stained tissue sections. The brilliance and sharpness of the stain without diffusion or unpredictable characteristics greatly facilitates diagnosis.

HEMATOXYLIN STAIN—PARAGON (aqueous alum hematoxylin). Made from our own formula. Yields vivid, sharply stained blue nuclei that are really blue—not off color or muddy. Extremely sharp staining and selective with no diffusion. Full bodied and strong. For a given staining time, repeatedly duplicates depth of staining from slide to slide—every day.

PS1101	Bottle (500 cc)	$2.25

EOSIN STAIN—PARAGON (alcoholic). A special eosin compound of our own preparation. Produces deep brilliant red counterstains. Packed in two forms—ready to use and concentrated (requiring the addition of 3 parts of 95% alcohol).

PS1201D	Bottle (500 cc) ready to use	$2.25
PS1201	Bottle (250 cc) for 1000 cc	3.00

ELASTIC FIBER STAIN—PARAGON. Our own resorcin-fuchsin modification of Weigert's Elastic Fiber Stain. Relieves the laboratory of the laborious work involved in the preparation of this important stain. Stains sharply with no diffusion into other tissue components.

PS1225	Bottle (250 cc)	$2.65

VAN GIESON STAIN—PARAGON. Especially designed to produce brilliant differential counterstaining with less tendency to wash out in rinsing alcohols.

PS1250	Bottle (250 cc)	$1.50

PARAGON MULTIPLE STAIN FOR FROZEN SECTIONS. Invaluable to the Pathologist where seconds count and the Surgeon waits for the diagnosis. A single solution which stains instantaneously yielding a hematoxylin-eosin like picture. No special technic. With Paragon Mounting Medium For Frozen Sections (water soluble) section is stained, mounted and under microscope in less than one minute.

PS1301	Paragon Multiple Stain For Frozen Sections	Bottle (50 cc)	$2.00
P451	Paragon Mounting Medium For Frozen Sections	Bottle (25 cc)	.50

Request samples on your institution letterhead.
Write for fully descriptive catalog number 1049 A which includes a descriptive section on staining technics.

All prices F. O. B. New York, New York, subject to change without notice.

Manufactured exclusively by

PARAGON C. & C. CO., Inc. • 2540 Belmont Ave., New York 58, N. Y.

Cable Address: Wijeno, New York.

Write for details on the following Paragon Staining Solutions:

ACID FAST BACTERIA STAIN • CRYSTAL VIOLET STAIN • GRAM'S IODINE SOLUTION
SAFRANIN STAIN • LOEFFLER'S ALKALINE METHYLENE BLUE • ZIEHL-NEELSEN STAIN
WRIGHT'S STAIN • BUFFER SOLUTION FOR WRIGHT'S STAIN

Schedule of Rates

	Hotel	Single Bedrooms	Double or Twin Bedrooms
31.	Abbey, 151 West 51st Street	6.00	8.50-10.00
32.	Ambassador, Park Ave. & 51st St.	11.00-15.00	14.00-18.00
58.	Astor, Broadway & 44th St.	$ 7.00-10.00	$11.00-15.00
8.	Barbizon (For Women) 140 East 63rd St.	4.75- 5.50	8.00- 8.50
12.	Barbizon-Plaza, 101 West 58th St.	6.50- 8.50	11.00-14.50
47.	Barclay, 111 East 48th St.	Suites only	
62.	Beaux Arts, 310 E. 44th St.	6.50- 8.50	8.50-12.00
43.	Beekman Tower, First Ave. & 49th St.	6.00- 8.00	7.00-12.50
1.	Bretton Hall, Broadway & 86th St.	4.00- 6.00	6.00- 7.00
45.	Bristol, 129 West 48th St.	....	7.00- 8.50
46.	Chatham, Vanderbilt Ave. & 48th St.	6.50-11.00	14.00-16.00
65.	Claridge, Broadway & 44th St.	6.00- 8.00	8.00-11.00
70.	Commodore, Lexington Ave. & 42nd St.	6.50- 9.50	10.50-14.00
64.	Dixie, 230 West 43rd St.	5.00- 6.00	8.00- 9.50
11.	Essex House, 160 Central Park South	8.00-13.00	11.00-17.00
80.	Governor Clinton, Seventh Ave. & 31st St.	6.00- 7.50	8.50-12.00
83.	Gramercy Park, Lexington Ave. & 21st St.	5.00- 9.00	7.00-12.00
19.	Great Northern, 118 West 57th St.	6.00- 7.00	8.00-10.00
84.	Grosvenor, 35 Fifth Ave.	7.00- 9.00	10.00-12.50
18.	Henry Hudson, 353 West 57th St.	5.00- 7.00	8.00-12.00
60.	Knickerbocker, 120 West 45th St.	....	8.00
53.	Luxor Baths (For Men) 121 West 46th St.	4.50	4.25
76.	McAlpin, Broadway & 34th St.	6.00- 9.50	8.00-13.00
16.	Madison, 15 E. 58th St.	6.50- 8.50	8.50-12.00
79.	Martinique, Broadway & 32nd St.	5.50- 7.50	8.00-12.00
75.	New Yorker, Eighth Ave. & 34th St.	5.50-10.00	9.00-16.00
52.	Paramount, 235 West 46th St.	7.00	9.00-10.00
21.	Park Sheraton, Seventh Ave. & 56th St.	5.00-10.50	7.50-13.50
55.	Piccadilly, 227 West 45th St.	6.00- 7.00	8.00-10.00
14.	Plaza, Fifth Ave. & 59th St.	10.00-14.00	14.00-24.00
49.	President, 234 West 48th St.	5.00- 6.00	6.00- 8.00
82.	Prince George, 14 East 28th St.	5.00- 8.00	7.50-10.50
13.	St. Moritz, 50 Central Park South	Suites only	
15.	Savoy Plaza, Fifth Ave. & 58th St.	11.00-16.00	15.00-20.00
73.	Shelburne, Lexington Ave. & 37th St.	8.00-10.00	10.00-14.00
48.	Shelton, Lexington Ave. & 49th St.	6.00-11.00	8.00-14.00
78.	Statler, Woman's Auxiliary Hdqtrs. Seventh Ave. & 33rd St.	6.00-10.50	9.00-17.00
25.	Sutton, 330 East 56th St.	4.50	....
35.	Taft, Seventh Ave. & 50th St.	4.75- 8.75	9.50-12.50
77.	Vanderbilt, Park Ave. & 34th St.	6.50- 8.50	10.00-12.00
40.	Waldorf Astoria (Headquarters Hotel, no rooms available)		
6.	Westbury, Madison Ave. at 69th St.	....	16.00
66.	Woodstock, 127 West 43rd St.	....	8.00-12.50
54.	Grand Central Palace	Location of exhibits	

Rates subject to 3% New York City tax on hotel rooms

102nd A.M.A.

Annual Meeting

New York City
June 1-5

New York City in June, 1953 will be the medical capital of the world. Visitors from every country will be attending the A. M. A. meeting. It will provide you a fruitful source of new information on the advances of scientific medicine.

Plan now for your attendance at this outstanding meeting. You will find a broad variety of subjects covered . . . and there will be ample opportunity for discussion of your problems with demonstrators in the scientific exhibits.

Remember, there is no registration fee, but be sure to do two things . . . register in advance and make your hotel reservation. Use the forms on these pages and mark your calendar so that you'll be in New York June 1-5, 1953.

Register in advance using this application

Please fill out this coupon in full and return it at once to the American Medical Association, 535 North Dearborn Street, Chicago 10, Illinois, and receive your registration identification card for the New York Meeting.

I,.....

Please write plainly or print your name

.....

(Office address—Street, City, Zone and State)

do hereby declare that I am a Member of the .....State Medical Association

I will be accompanied by.....

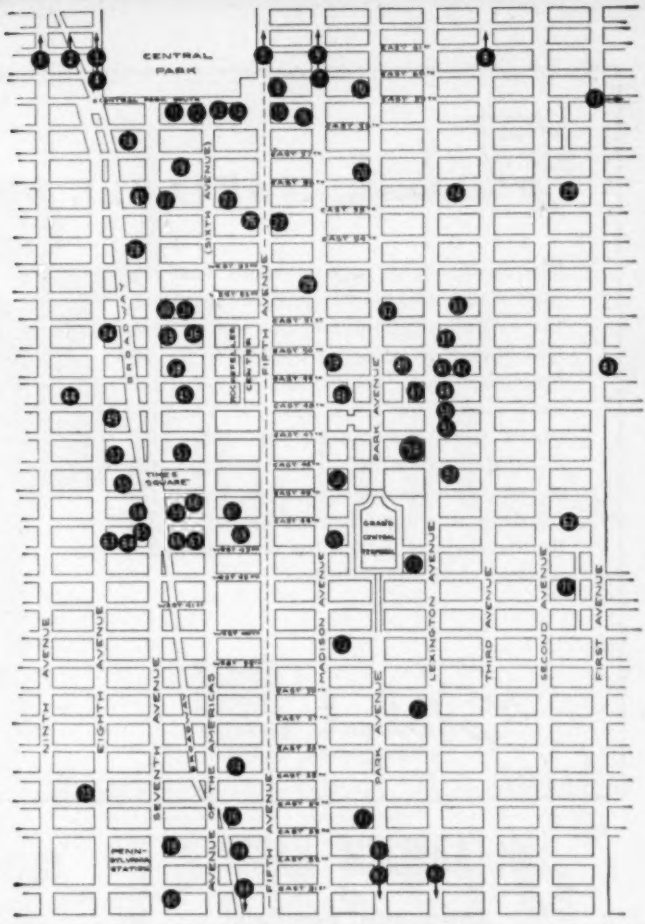
.....

Full name of each guest. Please do not include a physician as a guest. Every doctor must register in his own name.

IMPORTANT: All reservations for rooms must be cleared with the local subcommittee on hotels.

Contacting individual hotels will be useless as your application will in any case be referred to the subcommittee. Use the form at the bottom of this page addressing the Chairman, American Medical Association, Subcommittee on Hotels, 500 Park Avenue, New York 22, New York

A. M. A. Section
on
PATHOLOGY & PHYSIOLOGY
will meet in the
BELMONT PLAZA HOTEL
Make your room reservation
there or nearby.



Make your hotel reservation now!

APPLICATION FOR HOTEL ACCOMMODATIONS

Be sure to give four choices of hotels

Edward P. Flood, M.D.
Chairman, American Medical Association
Subcommittee on Hotels
500 Park Avenue, New York 22, N. Y.

Please reserve the following:

Hotel..... Hotel.....
First Choice Third Choice

Hotel..... Hotel.....
Second Choice Fourth Choice

.....Room(s) with bath for.....person(s). Rate $.....to $.....per room
Please check whether you prefer a double.....or twin.....bedroom
.....Room(s) and Parlor for.....person(s). Rate $.....

Arriving New York.....hour.....A.M. P.M. Leaving.....

Note: You will receive confirmation direct from the hotel accepting the reservation when made.

Rooms will be occupied by:
(Please attach list of additional names if you do not have sufficient space here.)

.....
Name **Street Address** **City** **Zone** **State**

.....

.....

If you are a Technical Exhibitor, be sure to give name of firm and individuals to occupy room or rooms reserved.

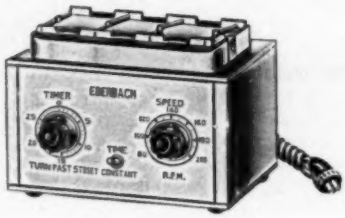
Signature.....

Mailing Address.....

City.....*Zone*.....*State*.....

Eberbach VARIABLE SPEED ROTATOR

Eberbach Slide Agglutination Test Rotators are available in three models: (1) Constant speed for the Kline Test; (2) Variable speed adaptable to the Boerner-Jones-Luken, Mazzini, cardiolipin and other tests; and (3) Variable speed platform model for slides, for mixing donor's blood as received, for blood typing and grouping, and for solution mixing where a swirling motion is desired. Write for Bulletin 210-S giving complete details.



Variable Speed Rotator above carries three 75 by 50 mm. slides. At end of operating period rotator stops, and the bell rings. Anodized aluminum case measures 8½ by 7 by 7 inches. Operates from 115 volt A.C. Cat. No. 21-146 is $80.00.

Eberbach CORPORATION
ANN ARBOR, MICH.

METABOLISM CAGE
for dogs and monkeys

30" x 30" x 28"d.

One of many styles of double and single deck cages. Heavy galvanized sheet metal, galvanized floor and pan.

NOTE: Slope of sides over floor for good drainage and sanitation.

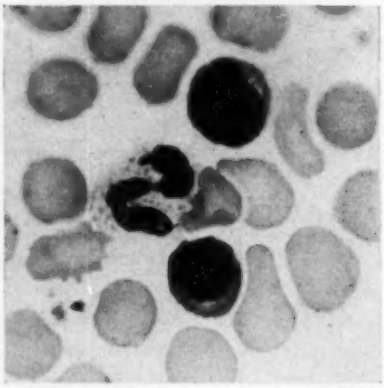
WRITE FOR NEW CAGE BOOKLET AND PRICES

BALTIMORE WIRE and IRON WORKS

510-512 NORTH JASPER • BALTIMORE 1, MARYLAND

CLINICAL PHOTOGRAPHY

An Established Service To The Medical Profession



- Surgical Drawings
- Photomicrography
- Photographs of Patients
- X-Ray Prints
- Copies from Books
- Lantern Slides
- Mouloges
- Motion Pictures
- Color and Black and White

Prompt Mail Orders

Martin Haggett
220 W. 42nd Street, New York 18, N.Y.
Telephone, Wisconsin 7-2602

Simple
Precise

Control...

of Prothrombin Time with

Solu-Plastin®

(THROMBOPLASTIN SOLUTION - SCHIEFFELIN)

SOLU-PLASTIN gives you these extras . . .

SIMPLE—Solu-Plastin is simple and easy to use. No extra work of preparation is required because it is supplied as a stable solution.

PRECISE—Prothrombin times are accurate, consistent and reproducible.

ECONOMICAL—Only the actual amount needed is used. No waste.

STANDARDIZED—**Every rigidly controlled lot** is standardized against normal and dicumarolized human plasma.

Supplied: 10 cc. bottles (with the same amount of 0.0125M Calcium Chloride Solution)

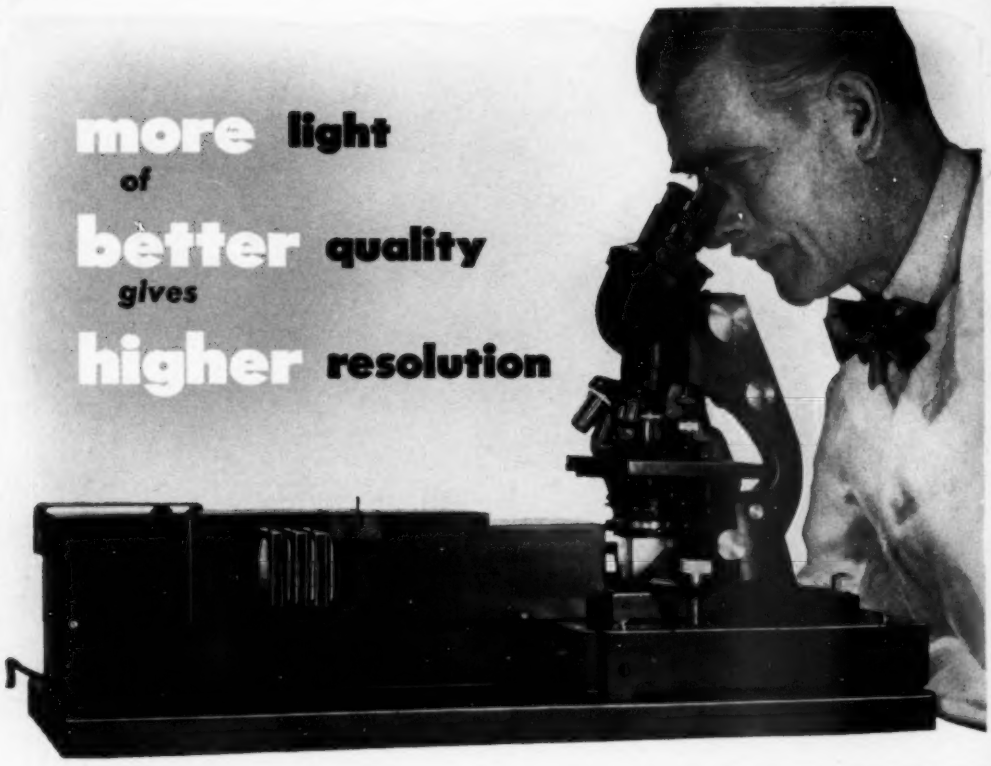
Schieffelin 1794 Quality Integrity Service

Write for literature and ***LARGE DIRECTION CARDS.***

Schieffelin & Co.

since 1794
pharmaceutical and research laboratories
18 Cooper Square, New York 3, N. Y.

more light of better quality gives higher resolution



Here is a light source we believe really worthy of the optical efficiency of the modern microscope. It achieves the long-sought-after state of critical illumination at all powers focused precisely upon the slide. The light enters the microscope objective at an uncommonly wide angle (i.e. high numerical aperture) providing resolution of the highest order.

Your first look at a slide so ideally illuminated will be a memorable experience. Let us send you the brochure describing this notable contribution to better microscopy.

Scopicon

MICROSCOPE LAMP

SCOPICON, INC. 215 EAST 149th STREET, NEW YORK 51, N. Y.

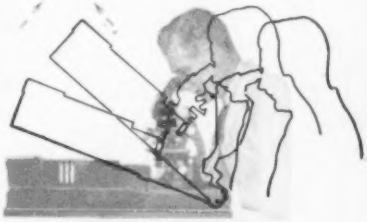
YOU *SEE* THE DIFFERENCE . . .

USUAL NARROW ANGLE

To fill objective lens, light must be sharply deflected when striking minute components under observation. Those incapable of widely deflecting the beam are not seen clearly.

SCOPICON WIDE ANGLE

Lens is filled by wide-angled beam. Diminished deflection required. Small detail sharply visible.



LOCKED IN PERMANENTLY ACCURATE RELATIONSHIP

The microscope is permanently clamped on the lamp platform; once the initial aligning adjustment has been made, it never changes. Carry the unit anywhere; tilt it to the steepest angle, if you will; optical alignment stays fixed always.